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Use of ADAM 12 for diagnosis and therapy of preeclampsia

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Use of ADAM 12 for diagnosis and therapy of preeclampsia

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The present invention relates to the use of ADAM 12, particularly ADAM 12-S, for

10   diagnosis and therapy of preeclampsia and related syndromes.

Preeclampsia (also known as toxemia or eclampsism) is a disorder or syndrome affecting about 5 % of pregnant women. It becomes manifest in the last trimester of pregnancy and is characterized by high blood pressure, protein in the urine, and – sometimes – swelling

15   (edema) of the face and hands. Preeclampsia may also include sudden weight gain, blurred vision, severe headaches, dizziness, and stomach pain.

In preeclampsia, the trophoblast (embryonic tissue that invades the uterus wall to form the embryonic part of the placenta) is poorly differentiated and shows incomplete invasion of

20   the uterus wall.

When left untreated, preeclampsia can lead to severe problems, endangering both mother and fetus. High blood pressure constricts the blood vessels in the uterus that supply the fetus with oxygen and nutrients. The fetal growth is retarded. Preeclampsia can increase

25   the risk of placental abruption (separation of the placenta from the uterus wall) before delivery, risk of kidney failure, cerebral hemorrhage, intravascular coagulation, lung edema, and circulatory collapse.

Furthermore, untreated preeclampsia can lead to a related life-threatening condition,

30   eclampsia. Eclampsia can lead to convulsions and coma. The HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome is also frequently considered to be related to preeclampsia, as preeclampsia is thought to increase the risk for HELLP. HELLP is characterized by destruction of red blood cells, liver damage, and a reduction in the number of blood platelets. Thus, also HELLP can be life-threatening.

Intrauterine growth retardation (small for gestational age (SGA) infants) is another syndrome related to preeclampsia, as it is associated with poor trophoblast differentiation and growth retardation of the embryo.

5

Pregnancy induced hypertension (PIH) is also a syndrome related to preeclampsia. It is characterized by high blood pressure, but lacks the proteinuria characteristic for preeclampsia.

10   Other syndromes related to preeclampsia are superimposed gestosis ("Aufpropfgestose") and gestational diabetes.

A causal treatment of preeclampsia and said related syndromes is not available. The usual treatment consists of the treatment of hypertension, intravenious magnesia to prevent
15   eclamptic convulsions and corticosteroids in case of HELLP syndrome. Very often symptoms can not be controlled and preterm delivery is the only way to save the life of mother and child. Preterm delivery is still associated with a high morbidity and mortality of the child.

20   Currently, diagnosis of preeclampsia is limited to relatively late arising symptoms such as blood pressure (typically above 140/90 mm Hg) and protein content of the urine (proteinuria, typically more than 0.3 g/24 hours or a level of 2+ by dipstick test). Another sign for preeclampsia is glomerular endotheliosis, a histological change of the kidney which can be observed in kidney biopsies of 50% of the patients. These markers are
25   relatively unreliable or become manifest only at later stages of preeclampsia. Similarly, said related syndromes can not be diagnosed in a satisfactory manner. In summary, there remains an urgent need for molecular markers indicative of preeclampsia (Beaulieu, M.D., Prevention of preeclampsia. In: Canadian Task Force on the Periodic Health Examination. Canadian Guide to Clinical Preventive Health Care. Ottawa: Health Canada, 1994; 136-43).

30

A small number of genes has been reported to be either upregulated (EPAS-1/HIF-2α, neurokinin B, TIMP-1, VEGFR-1 (=sFLT-1), VEGF, IGFBP-1, IGFBP-3, matrix metalloproteinase-2, leptin, plasminogen activator inhibitor 1 (PAI-1)) or downregulated (IGF-1, angiopoetin-2, decorin, various ephrins, PIGF, HLA-G) during preeclampsia.
35   PIGF has been reported to be downregulated in blood serum, but not in placental tissue. HB-EGF, various ephrins, and TGF-β3 have also been discussed as being de-regulated during preeclampsia.

However, most of these genes are only weakly up- or downregulated and/or can only be measured in placental tissue, requiring to take placenta tissue samples for analysis. Thus, frequent measurements at short time intervals are not possible. In any case, none of the genes cited above have been put into practice as routine markers for preeclampsia or related syndromes.

Consequently, a reliable diagnosis of preeclampsia or related syndromes is not possible at the moment. However, reliable diagnosis of onset and progression of said disorders or syndromes would allow to develop better treatment and to evaluate the risk for mother and fetus at each stage of pregnancy. Reliable diagnosis is also needed, because women experience no obvious symptoms at early stages of preeclampsia or related syndromes.

Therefore, there is a persistent need for molecular markers of preeclampsia or related syndromes. Such markers would also be useful to identify and classify different subtypes of preeclampsia and related syndromes.

Consequently, the problem underlying the present invention resides in improving diagnosis of preeclampsia or related syndromes. In particular, the problem resides in providing a marker more indicative of preeclampsia and/or related syndromes than other markers. In particular, the problem resides in providing a highly indicative marker that can also be measured in body fluids.

Another problem underlying the present invention resides in providing treatment of preeclampsia and/or related syndromes.

With respect to diagnosis, the problem is solved by use of a nucleic acid molecule comprising a nucleic acid selected from the group consisting of

a) a nucleic acid with a sequence as presented in SEQ ID NO: 1 or 3;

b) a nucleic acid with a sequence that exhibits a sequence identity with any of the sequences defined in a) of at least 70% over 300 residues;

c) a nucleic acid which is capable of hybridizing with the nucleic acid as defined in a), or b) under conditions of medium or high stringency;

d) a nucleic acid with the antisense-sequence of any of the sequences defined in a), b) or c);

e) a fragment of any of the nucleic acids as defined in a), b), c), or d);

f) an RNA corresponding to any of the sequences defined in a), b), c), d) or e);

for diagnosis of preeclampsia or a related syndrome.

5   The problem is also solved by use of a ligand binding specifically to a peptide or polypeptide with a sequence selected from the group consisting of

a) an amino acid sequence as presented in SEQ ID NO: 2 or 4;
b) an amino acid sequence exhibiting a sequence identity with any of the
10   amino acid sequences according to a) of at least 85% over 100 amino acid residues;
c) a fragment of any of the sequences defined above

for diagnosis of preeclampsia or a related syndrome.

15

For reference, the individual SEQ ID NO denotes the following sequence:

SEQ ID NO: 1	nucleotide sequence of the human ADAM 12-L cDNA
SEQ ID NO: 2	protein sequence of the human ADAM 12-L protein
SEQ ID NO: 3	nucleotide sequence of the human ADAM 12-S cDNA
SEQ ID NO: 4	protein sequence of the human ADAM 12-S protein
SEQ ID NO: 5	nucleotide sequence region specific for the human ADAM 12-L cDNA
SEQ ID NO: 6	protein sequence region specific for human ADAM 12-L
SEQ ID NO: 7	nucleotide sequence region specific for the human ADAM 12-S cDNA
SEQ ID NO: 8	protein sequence region specific for human ADAM 12-S
SEQ ID NO: 9	PCR primer (1), ADAM 12 5' primer
SEQ ID NO: 10	PCR primer (2), ADAM 12-L 3' primer
SEQ ID NO: 11	PCR primer (3), ADAM 12-L 5' primer
SEQ ID NO: 12	PCR primer (4), ADAM 12-L 3' primer
SEQ ID NO: 13	PCR primer (5), ADAM 12-S 3' primer, containing a single nucleotide exchange (G instead of C) at position 15.
SEQ ID NO: 14	PCR primer (6), ADAM 12-S 3' primer, correct sequence
SEQ ID NO: 15	PCR primer (7), ADAM 12-S 5' primer
SEQ ID NO: 16	PCR primer (8), ADAM 12-S 3' primer
SEQ ID NO: 17	PCR primer (9), human cyclophilin B 5' primer

The numbers 10, 20, 25, 30, 35 appear in the left margin adjacent to the corresponding lines.

SEQ ID NO: 18   PCR primer (10), human cyclophilin B 3' primer
SEQ ID NO: 19   PCR primer (11), EF-1α 5' primer
SEQ ID NO: 20   PCR primer (12), EF-1α 3' primer

SEQ ID NO: 1 to 16 refer to nucleic acids, peptides or polypeptides derived from ADAM 12. ADAM 12 (meltrin alpha) is a member of the ADAM (a disintegrin and metalloprotease) family. Human ADAM 12 exists in two isoforms, a longer membrane anchored isoform, ADAM 12-L (SEQ ID NO: 1, 2, GenBank Acc. No. AF023476) and a shorter secreted isoform, ADAM 12-S (SEQ ID NO: 3, 4, GenBank Acc. No. AF023477).

ADAM 12 has been implicated in cell adhesion and muscle cell differentiation and fusion. Additionally, ADAM 12 has been implicated in fat tissue development and it is upregulated in several types of human carcinomas. ADAM 12-S is expressed highly in the placenta and can be detected in blood serum during pregnancy, while it is undetectable in non-pregnancy serum. However, no connection of ADAM 12, particularly ADAM 12-S, to preeclampsia or a related syndrome has been reported previously.

As a consequence of the present invention it is known that expression, particularly transcription, of ADAM 12 is strongly upregulated in preeclampsia. In a comparison with 1600 known genes examined by gene array analysis, ADAM 12 was found to be the most strongly upregulated gene (Example 8). Therefore, ADAM 12 can be considered a valuable diagnostic marker, and upregulation of ADAM 12 can be used to diagnose preeclampsia and related syndromes. ADAM 12, particularly ADAM 12-S, is present in body fluids such as blood. Blood samples are taken routinely at physical examinations during most pregnancies. Thus, it is possible to routinely monitor pregnant mothers at short intervals for upregulation of ADAM 12, allowing to early detect and monitor preeclampsia or related disorders with minimal effort and risk for the mother.

"Nucleic acid" according to the present invention relates to all known nucleic acids such as DNA, RNA, peptide nucleic acids, morpholinos, and nucleic acids with backbones other than phosphodiesters, such as phosphothioates or phosphoramidates. The person skilled in the art knows which nucleic acids are best suited for a particular purpose, such as diagnosis, or a particular method (such as Northern hybridization or polymerase chain reaction (PCR)).

Unless otherwise specified, the manipulations of nucleic acids and polypeptides/-proteins can be performed using standard methods of molecular biology and immunology (see, e.g. Maniatis et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY; Ausubel, F.M. et al. (eds.) "Current protocols in Molecular 5 Biology". John Wiley and Sons, 1995; Tijssen, P., Practice and Theory of Enzyme Immunoassays, Elsevier Press, Amsterdam, Oxford, New York, 1985).

The term "comprise" preferably refers to nucleic acid molecules in which the nucleic acids with the described sequences are functionally relevant, e.g. for diagnostic use or 10 therapeutic use, such as vectors for therapeutical use or expression of corresponding RNAs or proteins. For example, the above defined nucleic acids may be part of a vector allowing transcription of antisense DNA or RNA probes. Preferably, any additional nucleic acids upstream or downstream of the nucleic acid are not longer than 80 kb, more preferably not longer than 20kb, more preferably not longer than 5 kb. More preferably, the term 15 "comprise" does not relate to large constructs accidentally including the sequence, such as genomic BAC or YAC clones. More preferably, the nucleic acid molecule consists of the above defined nucleic acids.

Preferably, the nucleic acid molecule is purified and/or isolated. Suitable purification 20 methods, including gel electrophoresis and capillary electrophoresis, are well known in the art. An isolated nucleic acid molecule is understood as a nucleic acid molecule isolated from the human or animal body or gained by means of a technical process, such as a microbiological process or organic synthesis.

25 In a preferred embodiment, said nucleic acid molecule comprises a nucleic acid with a sequence selected from the group of sequences as presented in SEQ ID NO: 1 or 3. SEQ ID NO: 1 corresponds to the sequence presented in GenBank Acc. No. AF023476, and SEQ ID NO: 3 corresponds to the sequence presented in GenBank Acc. No. AF023477.

30 In another preferred embodiment, said nucleic acid molecule comprises a nucleic acid with a sequence that exhibits a sequence identity with any of the sequences defined above of at least 70%, preferably at least 80%, more preferably at least 90%, and most preferred of at least 95% over 300 nucleotide residues.

"Sequence identity" refers to the degree of indentity (% identity) of two sequences that in the in the case of peptides or polypeptides can be determined by means of for example BLASTP 2.0.1 and in the case of nucleic acids can be determined by means of for example BLASTN 1.014, wherein the Filter is set off and BLOSUM is 62 (Altschul et al., Nucleic
5   Acids Res., 25:3389-3402).

Such nucleic acids include those whose sequences deviate from the above defined sequences due to the degeneration of the genetic code, the presence of different alleles in the human or animal population, and the presence of related sequences in different species.
10   Such nucleic acids can still be used according to the present invention.

In another preferred embodiment, the nucleic acid molecule comprises a nucleic acid capable of hybridizing with the nucleic acids according to SEQ ID NO: 1 or 3 under conditions of low, preferably medium, or, more preferred, high stringency.
15

In such hybrids, duplex formation and stability depend on substantial complementarity between the two strands of the hybrid and a certain degree of mismatch can be tolerated. Therefore, the nucleic acids and nucleic acid probes of the present invention may include mutations (both single and multiple), deletions, insertions of the above-identified
20   sequences, and combination thereof, as long as that nucleic acids still have substantial sequence similarity to the original sequence which permits the formation of stable hybrids with the target nucleotide sequence of interest. For example, a single nucleotide mismatch in PCR primer (SEQ ID NO: 13) as compared to the correct primer sequence (SEQ ID NO: 14) was found to be tolerable.
25

Suitable experimental conditions for determining whether a given DNA or RNA sequence "hybridizes" to a specified polynucleotide or oligonucleotide probe involve presoaking of the filter containing the DNA or RNA to examine for hybridization in 5 x SSC (sodium chloride/sodium citrate) buffer for 10 minutes, and prehybridization of the filter in a
30   solution of 5 x SSC, 5 x Denhardt's solution, 0,5 % SDS and 100 mg/ml of denaturated sonicated salmon sperm DNA (Maniatis et al., 1989, see above), followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random primed (Feinberg, A.P. and Vogelstein, B. (1983), Anal. Biochem. 132:6-13), ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/µg) probe for 12 hours at approximately 45°C. The filter is then
35   washed twice for 30 minutes in 2 x SSC, 0,5% SDS at at least 55°C (low stringency), at

least 60°C (medium stringency), preferably at least 65°C (medium/high stringency), more preferably at least 70°C (high stringency) or most preferably at least 75°C (very high stringency). Molecules to which the probe hybridizes under the chosen conditions are detected using an x-ray film or a "phosphor imager".

5

In another preferred embodiment, said nucleic acid molecule comprises a nucleic acid which is capable of hybridizing with the nucleic acids according to SEQ ID NO: 1 or 3, but not capable of hybridizing with nucleic acids encoding ADAM 19 or ADAM 33.

10   Preferably, the nucleic acids should be chosen from sequence regions specific for the above-defined sequences, i.e. the nucleic acids should not cross-hybridize with nucleic acids encoding other genes, particularly other metalloprotease genes, more particularly ADAMs (Seals, D.F, and Courtneidge, S.A. (2003). The ADAMs family of metalloproteases: multidomain proteins with multiple functions. Genes & Dev. 17, 7-30),
15   even more particularly ADAM 19 (GenBank Acc. No. Y13786) or ADAM 33 (GenBank Acc. No. AB009672, most particularly ADAM 2 (fertilin beta, GenBank Acc. Nos. AJ133005 or NM_001464) (see also Yoshinaka, T., Nishii, K., Yamada, K., Sawada, H., Nishiwaki, E., Smith, K., Yoshino, K., Ishiguro, H., and Higashiyama, S. (2002). Identification and characterization of novel mouse and human ADAM 33s with potential
20   metalloprotease activity. Gene 282, 227-236), under the applied conditions, preferably conditions of medium, medium/high, high or very high stringency.

In another preferred embodiment, the nucleic acid molecule comprises a nucleic acid with the antisense-sequence of any of the sequences defined above.

25

In another preferred embodiment, the nucleic acid molecule comprises a nucleic acid which is a fragment of any of the nucleic acids as defined above.

The term "fragment" as used according to the present invention can have different
30   meanings depending on the molecule and purpose referred to. A person skilled in the art knows how to choose appropriate fragments for the relevant purpose. In particular, the person skilled in the art knows how to choose fragments of appropriate length and suitable sequence (see also Examples 9 and 10).

35   Preferably, the fragments should be chosen from sequence regions specific for the above-defined sequences, i.e. the chosen fragments should not cross-hybridize with nucleic acids

encoding other genes, particularly other metalloprotease genes, more particularly ADAMs, even more particularly ADAM 19 or ADAM 33, most particularly ADAM 2, under the applied conditions. Sequence regions specific for the above defined sequences can be determined easily by the person skilled in the art, e.g. by database searches and sequence
5   comparisons. Such searches and comparisons are routine in the art and possible using sequence analysis programs such as BLAST and ClustalW. Preferably, the fragments should be chosen from regions of high complexity which can be determined by low complexity filters available in standard sequence analysis programs. Specificity for the purpose of the present invention may also be achieved in spite of cross-hybridization, if the
10   investigated sequences can still be measured unequivocally, e.g. by the size of amplified PCR products. Therefore, such fragments, particularly PCR primers, can still be used according to the present invention.

In another preferred embodiment, the fragment has a sequence consisting of or being part
15   of any of SEQ ID NO: 5, 7, 9, 10, 11, 12, 13, 14, 15, 16;

In another preferred embodiment, the fragment should be at least 12, preferably at least 15, more preferably at least 19, even more preferably at least 30 nucleotides in length.

20   In another preferred embodiment, said nucleic acid molecule comprises an RNA corresponding to any of the sequences defined above. RNA molecules are frequently used as probes for analysis of nucleic acids and measurement of gene expression, e.g by in situ hybridization or RNAse protection assays. A suitable RNA in situ hybridization probe may, for example, comprise or consist of the 667 nucleotide sequence fragment
25   corresponding to the antisense sequence of position 1769 to 2445 in SEQ ID NO: 3.

Diagnosis according to the present invention includes monitoring, confirmation, subclassification and prediction of the relevant disorder or syndrome. Monitoring relates to monitoring of an already diagnosed disorder, e.g. to analyze the progression of the disorder
30   or the influence of a particular treatment on the progression of disorder. Confirmation relates to the confirmation or substantiation of a diagnosis already performed using other indicators, symptoms, or markers. Subclassification relates to further defining a diagnosis according to different subclasses of the diagnosed disorder, e.g. defining according to mild and severe forms of the disorder. Prediction relates to predicting a disorder before other
35   symptoms or markers have become evident or have become significantly altered. Prediction also relates to classifying patients into groups of low, medium, or high risk of developing the disorder.

In another preferred embodiment, diagnosis relates to the diagnosis of syndromes related to preeclampsia.

5 According to the present invention, syndromes "related" to preeclampsia include other pregnancy-associated disorders or syndromes with similar aetiology and/or symptoms as preeclampsia. Particularly included are eclampsia, pregnancy induced hypertension, HELLP syndrome, intrauterine growth retardation and superimposed gestosis. Most particularly, related syndromes are eclampsia, pregnancy induced hypertension, HELLP
10 syndrome, and superimposed gestosis.

For diagnosis of preeclampsia or related syndromes, preferably the expression level or amount of ADAM 12 RNA or its corresponding protein is determined. The term "measuring" according to the present invention relates to determining the amount or
15 activity, preferably semi-quantitatively or quantitatively, of the substance in question. Measuring is frequently done indirectly, through measuring of ligands, labels, or enzymatic reaction products.

ADAM 12 expression can be measured in tissue samples and body fluid samples. A tissue
20 sample refers to any kind of tissue obtained from the dead or alive human or animal body, particularly a placental tissue sample, more particularly a human placental tissue sample. Body fluid according to the present invention refers to any kind of body fluid, particularly to blood, amniotic fluid, lymphe, cerebral liquor, urine, and saliva, more particularly to blood, amniotic fluid, and urine, most particularly to blood. Expression according to the
25 present invention includes transcription, translation and transcript or protein stability.

RNA can be measured easily and quantitatively by methods known to the person skilled in the art. RNA can be obtained or isolated by purification methods known in the art, e.g. involving steps of enzymatic digestion of tissue or proteins, and
30 phenol/chloroform/isoamylalcohol extraction.

Suitable methods for measuring ADAM 12 expression, particularly ADAM 12 RNA (transcript), include the hybridization of nucleic acid probes, such as in situ hybridizations, Northern hybridizations, RNAse protection assays, gene arrays, RT-PCR, and other
35 suitable methods known in the art. Preferably, a method allowing quantitative or semi-quantitative measurement, such as quantitative RT-PCR, is chosen.

Characteristics of suitable nucleic acid probes, e.g. in situ hybridization probes or PCR primers, are known in the art. The probes may be based on DNA, RNA or derivatives thereof such as peptide nucleic acids. The probes may be synthesized by use of DNA synthesizers according to standard procedures or, preferably for long sequences, by use of

5   PCR technology with a selected template sequence and selected primers. Depending on the procedure, the nucleic acid probes may be labeled with any suitable substances known in the art, such as digoxigenin, biotin, or thyroxin (as targets of a secondary ligands, particularly a secondary antibody or a fragment thereof), luminescent or fluorescent labels (e.g. fluorescein) and radioactive isotopes (^{32}P, ^{125}I, ^{35}S, and the like). A probe labeled with

10 a radioactive isotope can be constructed from a DNA template by a conventional nick translation reaction or by randomly primed polymerization. The probe may also be labeled at both ends with different types of labels, for example with an isotopic label at one end and a biotin label at the other end. The labeled probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs.

15

Although gene transcripts are usually present only within cells, ADAM 12 RNA can also be measured (at lower levels) in body fluids, particularly in blood samples, including blood plasma and serum, by methods known in the art (Ng, E.K.O., Tsui, N.B.Y., et al. (2003). Messenger RNA of placental origin is readily detectable in maternal plasma. Proc Natl

20 Acad Sci USA, vol. 100(8):4748-53 and Ng, E.K.O., Leung, T.N., et al. (2003). The concentration of circulating Corticotropin-releasing hormone RNA in maternal plasma is increased in preeclampsia. Clin. Chem. 49, 727-731). According to the present invention, blood plasma is the fluid part of the blood, it lacks the corpuscular components such as blood cells and platelets. Blood serum is blood plasma additionally lacking fibrinogen.

25

Diagnosis is preferably performed in vitro, i.e. using elements isolated from the body. Tissue samples and body fluid samples can be obtained by laboratory assistants trained for this purpose during routine examinations. Body fluid and tissue samples can be obtained by any method known to the person skilled in the art, including amniocentesis and biopsy

30 (particularly chorion villi biopsy). In particular, blood samples are taken routinely at physical examinations during most pregnancies.

Routine examinations are typically performed in 4 week intervals during the first two trimenons, and biweekly during the last trimenon. Typically, blood samples are taken in

35 week 13 and at least once between weeks 21 and 28 of pregancy. However, many pregnant women come to examinations on a bi-weekly or weekly basis, particularly during the last half of pregnancy.

Thus, it is possible to routinely monitor pregnant women at short intervals for upregulation of ADAM 12, allowing to early detect and monitor preeclampsia or related disorders with minimal effort and risk for mother and fetus.

5

Levels (amounts) of ADAM 12 expression indicative or of diagnostic value for preeclampsia and its related syndromes can be detected by comparing expression levels under healthy conditions with expression levels under sick condititions (conditions of the existing or developing relevant disorder or syndrome). For example, the levels under sick

10   conditions can be determined relative to a healthy control group, or relative the mean population of pregnant mothers, or relative to earlier measurements for the same person.

In the present invention, approximately 4 to 5-fold increases of ADAM 12 transcript levels as measured by quantitative Real-Time RT-PCR were found to be correlated with

15   preeclampsia (see Example 10). The transcript levels were increased in placental tissue of both maternal and fetal origin.

Further levels of ADAM 12 expression indicative of preeclampsia and related syndromes can be determined in more detail in clinical screenings as known in the art. For example, a

20   level deviating from the average level (e.g. the mean level of the entire investigated group) by more than two standard deviations, preferably by more than three standard deviations may be considered indicative of the analyzed disorder. Deviations indicative of preeclampsia or a related syndrome may be even smaller, if additional markers indicative of the disorder are also deviating from the average level. More weakly deviating levels

25   may be used to classify a person into a medium or high risk group of developing the disorder. Those markers may include blood pressure, protein content in the urine and molecular markers. Additional factors may influence the diagnosis, e.g. the regular blood pressure or the number of previous pregnancies in a given patient. Usually, the highest risk for preeclampsia is during the first pregnancy. Thus, measuring ADAM 12 expression

30   yields a diagnostic parameter that is preferably compared with additional parameters to allow a decision about a necessary treatment.

The present invention also relates to a method of diagnosis of preeclampsia in which the above defined nucleic acid molecules are used.

35

In another preferred embodiment, the fragment has a sequence consisting of or being part of SEQ ID NO: 7, 13, 14, 15, 16.

In another preferred embodiment, the fragment has a sequence consisting of or being part of SEQ ID NO: 5 or 10, 11, 12.

5   SEQ ID NO: 7, 13, 14, 15, and 16 are unique (specific) to the splice variant ADAM 12-S. SEQ ID NO: 5 or 10, 11, 12 are unique (specific) to the splice variant ADAM 12-L. Both splice variants, ADAM 12-L and ADAM 12-S, are upregulated in preeclampsia. However, the splice variants show different regulation properties. In a control group, the level of ADAM 12-L RNA was found to be close to zero, whereas ADAM 12-S showed a
10   measurable basal expression level (both measured by semi-quantitative RT-PCR). Also, the total (upregulated) level of ADAM 12-L RNA in preeclampsia was lower than the one of ADAM 12-S RNA.

For specific detection of mRNA encoding the long splice variant, ADAM 12-L, or the
15 . short splice variant, ADAM 12-S, the probe should be directed against the sequence regions unique to these splice variants. For example, probes may be directed against the unique 3'-ends of ADAM 12-L (SEQ ID NO: 5) and ADAM 12-S (SEQ ID NO: 7). As known to the person skilled in the art, a short overlap of the probes with regions common to both splice variants may be tolerable without loss of specificity. For example, the 5'
20   ends of PCR primers may be located in sequence regions common to both transcripts. It is also sufficient if only one of the two primers is located in the regions unique to each splice variant. E.g., the primer according to SEQ ID NO: 9 may be used in combination with the primers according to SEQ ID NO: 13, 14, or 16 to amplify a product specific for ADAM 12-S.
25
As mentioned above, the invention also relates to a the use of a ligand binding a peptide or polypeptide as defined above.

Thus, the present invention also relates to the measurement of ADAM 12 protein. In
30   another preferred embodiment, the biological activity of ADAM 12 is measured. ADAM 12 protein or biological activity can be measured in tissue samples, e.g. in placental tissue samples or other samples taken from the patient.

ADAM 12, particularly ADAM 12-S, is also present in body fluids, particularly the blood
35   (Shi, Z., Xu, W., Loechel, F., Wewers, U.M., and Murphy, L.J. (2000). ADAM 12, a disintegrin metalloprotease, interacts with insulin-like growth factor-binding protein-3. J. Biol. Chem. 275, 18574-18580; Gilpin, B.J., Loechel, F., Mattei, M.-G., Engvall, E.,

Albrechtsen, R., and Wewer, U.M. (1998). A Novel, Secreted Form of human ADAM 12 (Meltrin alpha) Provokes Myogenesis in Vivo. J. Biol. Chem. 273, 157-166). Therefore, ADAM 12-S amount or level of biological activity can easily be measured in body fluids, particularly in blood, blood plasma or blood serum.

Biological activity according to the present invention relates to any activity exerted by ADAM 12, ADAM 12-L or ADAM 12-S in the human or animal body. In particular, biological activity relates to proteolytic activity, in particular to proteolytic activity exerted against HB-EGF, IGFBP-3, or IGFBP-5. According to the present invention, the term "biological activity" also relates to any effects, particularly undesired or toxic effects, exerted by ADAM 12, ADAM 12-L, or ADAM 12-S if they are more highly expressed or more active than usual.

In a preferred embodiment, the peptide or polypeptide may comprise a sequence selected from the amino acid sequences presented in SEQ ID NO: 2 or 4. SEQ ID NO: 2 corresponds to the sequence presented in GenBank Acc. No. AF023476 and SEQ ID NO: 4 corresponds to the sequence presented in GenBank Acc. No. AF023477.

In another preferred embodiment, said peptide or polypeptide has a sequence exhibiting a sequence identity with any of the amino acid sequences defined above of at least 85%, preferably 90%, more preferably 95% over 100 amino acid residues.

In another preferred embodiment, said peptide or polypeptide has a sequence which is a fragment of any of the amino acid sequences defined above.

The above defined peptide or polypeptide may also be part of fusion peptides or polypeptides. In such fusions another peptide or polypeptide may be fused at the N-terminus or the C-terminus of the peptide or polypeptide of interest. A fusion can be produced by fusing a nucleic acid sequence (or a portion thereof) encoding another polypeptide to a nucleic acid sequence (or a portion thereof) of the present invention. Techniques for producing fusion polypeptides are known in the art and include ligating the coding sequences so that they are in frame and the expression of the fusion polypeptide is under control of the same promotor(s) and terminator.

With respect to diagnosis, the definitions given above for the use of nucleic acids apply analogously.

Suitable methods for measurement of ADAM 12 are known to the person skilled in the art. For example, measurement can be done indirectly, through measuring of cellular responses, the amount of bound ligands, labels, or enzymatic reaction products.

5

For measuring cellular responses, the sample or processed sample is added to a cell culture and an internal or external cellular response is measured. The cellular response may include the expression of a reporter gene or the secretion of a substance, e.g. a peptide, polypeptide, or a small molecule.

10

Other preferred methods for measurement may include measuring the amount of a ligand binding specifically to the ADAM 12 proteins. Binding according to the present invention includes both covalent and non-covalent binding.

15 A ligand according to the present invention can be any peptide, polypeptide, nucleic acid, or other substance (including small molecules) binding to peptides, polypeptides with the above described sequences. It is well known that the above-defined peptides or polypeptides, if obtained or purified from the human or animal body, can be modified, e.g. by glycosylation. A suitable ligand according to the present invention may bind these
20 peptides or polypeptides also via such sites.

Preferably, the ligand should bind specifically to said peptides or polypeptides. "Specific binding" according to the present invention means that the ligand should not bind substantially to ("cross-react" with) another protein, isoform or substance present in the
25 sample investigated. Preferably, the specifically bound protein or isoform should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant protein, isoform, or substance. Other relevant proteins may include other ADAMs, particularly ADAM 2, more particularly ADAM 19 or ADAM 33.

30

Non-specific binding may be tolerable, particularly if the investigated ADAM 12 proteins or isoforms can still be distinguished and measured unequivocally, e.g. according to their size on a Western Blot, or by their relatively higher abundance in the sample.

Binding of the ligand can be measured by any method known in the art. Preferably, the method is semi-quantitative or quantitative. Suitable methods are described in the following.

5   If the ligand does not only bind, but also serves as a substrate of the above-defined peptides or polypeptides, the reaction product may be measured directly (e.g. cleaving of HB-EGF, IGFBP-3, or IGFBP-5 as a substrate may be measured by analyzing the cleaved product, e.g. on a Western Blot or by ELISA).

10   Furthermore, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand.

Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves

15   binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often advantageous to increase the signal. Suitable secondary and higher order ligands may

20   include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.)

The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin,

25   digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus.

Suitable labels are any labels detectable by an appropriate detection method. Typical labels

30   include include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels.

Enzymatically active labels include e.g. hydrogen peroxidase, alkaline phosphatase, and

35   derivatives thereof. Suitable substrates for detection of these enzymes include di-amino-benzidine (DAB), NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), and

CDP-Star™ (Amersham). Suitable enzyme-substrate combinations result e.g. in colored reaction products, fluorescence or chemoluminescence. Typical fluorescent labels include Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Typical radioactive labels include ^{35}S, ^{125}I, ^{32}P and the like.

Suitable measurement methods according the present invention also include electrochemiluminescence (electro-generated chemiluminescence), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, solid phase immune tests and 2D gel electrophoresis. If the position of the ADAM 12 protein (including its isoforms) in a 2D gel is known (e.g. identifiable by mass spectroscopy), then 2D gel electrophoresis may allow detection of ADAM 12 without use of a specifically binding ligand.

In another preferred embodiment, the peptide or polypeptide has a sequence which is selected from the sequence presented in SEQ ID NO: 8.

This embodiment relates to the isoform ADAM 12-S. ADAM 12-S is soluble and can be found at high concentrations in the blood. Therefore, ADAM 12-S can be measured easily using ligands binding to a peptide or polypeptide with a sequence according to SEQ ID NO: 4 and 8, particularly SEQ ID NO: 8. For specific detection of the short isoform (ADAM 12-S) or the long isoform (ADAM 12-L), binding of the ligand should be determined by regions unique to these isoforms, such as the C-terminal ends of ADAM 12-L (SEQ ID NO: 6) and ADAM 12-S (SEQ ID NO: 8). An example is an antibody raised against such a peptide or polypeptide. A suitable antibody has been described previously (Gilpin, B.J., Loechel, F., et al. (1998). A Novel, Secreted Form of Human ADAM 12 (Meltrin α) Provokes Myogenesis in Vivo. Journal of Biological Chemistry 273(1), 157-166). Said antibody is directed against a region (encoded by base pairs 2000-2433 according to Fig. 1 of Gilpin et al., 1998, cited above), which contains the first 4 amino acids (EARQ) of the C-terminal end unique to ADAM 12-S. However, in some samples, such as blood, ADAM 12-S is the most prevalent isoform and can be measured with a ligand directed against a region common to both isoforms.

In another preferred embodiment, the ligand is selected from the group consisting of

    a)   KB-R7785 or a derivative thereof;

b)   TIMP-1, TIMP-2, TIMP-3, IGFBP-3, IGFBP-5, HB-EGF, α_2-macroglobulin, PKC-δ, α-actinin-1, α-actinin-2, src, Grb-2, or syndecan-4;

c)   antibodies;

d)   nucleic acid or protein aptamers;

e)   fragments or derivatives of any of the substances defined in b), c), or d).

Several ligands binding to ADAM 12 are known in the art, including small molecules (KB-R7785 (Asakura, M., Kitakaze, M., Takashima, S., Liao, Y. (2002). Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: Metalloproteinase inhibitors as a new therapy. Nature Medicine 8: 35-40), polypeptides or proteins (TIMP-3, IGFBP-3, IGFBP-5, HB-EGF, α_2-macroglobulin), and antibodies (see also Gilpin, B.J., et al. (1998), cited above). A polyclonal antibody against ADAM 12 is commercially available from Serotec (Cat. No. AHP758). Another antibody, directed against the pro-domain of ADAM-12, is available from Biomol (Cat. No. SA-378). The pro-domain is a domain that is cleaved off during activation of ADAM-12. Methods to generate antibodies or aptamers are well-known in the art. Identification and production of suitable antibodies or aptamers is also offered by commercial suppliers.

Notably, HB-EGF and IGFBPs, such as IGFBP-3 and IGFBP-5, are not only ligands of ADAM 12, but also substrates of the ADAM 12 proteolytic activity.

The person skilled in the art is familiar with methods to develop derivatives of the ligands according to a) to d) with higher affinity or specificity. For example, random mutations can be introduced into the proteins or substitutions into other molecules. These derivatives can then be tested for binding according to the screening procedure described above.

The term "antibody" as used herein includes both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)$_2$ fragments that are capable of binding antigen or hapten. The present invention also includes "humanized" hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art.

In a preferred embodiment, the ligand is directed against a fragment of any of the substances defined in b), c), or d). It is well-known that binding of antibodies, nucleic acids, peptides, or polypeptides occurs via specific binding regions, so-called binding sites, epitopes, or binding pockets. Such binding regions can be determined by methods known

5   in the art, e.g. mutational analysis or NMR spectroscopy. Thus, fragments including only said binding regions can be determined. Those fragments may bind similarly or better than the original antibody, nucleic acid, peptide, polypeptide. Such fragments can also be used according to the present invention.

10   In a preferred embodiment, the above described use of nucleic acid molecules or ligands for diagnosis is done in conjunction with a diagnostic agent for the measurement of expression of any of the genes or proteins selected from the group consisting of (a) EPAS-1/HIF-2α, (b) neurokinin B, (c) TIMP-1, (d) VEGFR-1, (e) VEGF, (f) IGFBP-1, (g) IGFBP-3, (h) matrix metalloproteinase-2, (i) leptin, (j) PAI-1, (k) IGF-1, (l) angiopoetin-2,

15   (m) PIGF, (n) HLA-G, (o) HB-EGF, (p) TGFβ-3 (q) decorin, and/or a diagnostic tool for the measurement of blood pressure or protein content of the urine. Comparative results for some of these genes are shown in Example 8.

Suitable diagnostic agents for the measurement of expression of said genes or proteins are

20   known to the person skilled in the art. Suitable diagnostic agents can be defined analogously as above for ADAM 12. In particular, suitable diagnostic agents may comprise nucleic acids hybrizing to said genes or transcripts, antibodies, aptamers, and other ligands binding specifically to said proteins or the nucleic acids encoding said proteins.

25   Suitable antibodies are commercially available for most of the above defined proteins and can be found through public databases known to person skilled in the art, e.g. www. biocompare.com. Examples for suppliers are given in the following: EPAS-1/HIF-2α (Abcam, Novus-Biologicals), neurokinin B (United States Biological), TIMP-1 (Affinity BioReagents (ABR)), VEGFR-1 (Chemicon, Lab Vision, Upstate, CalTag), VEGF

30   (Anogen, Assay Designs Inc., BD Biosciences Pharmingen, Chemicon, Novus Biologicals, and others), IGFBP-1 (Cell Sciences, Novus Biologicals), IGFBP-3 (BD Biosciences Pharmingen, Novus Biologicals), matrix metalloproteinase-2 (Affinity BioReagents (ABR), Chemicon, Lab Vision), leptin (Affinity BioReagents (ABR), BD Pharmingen, BioSource International, Chemicon, Peprotech, and others), IGF-1 (Novus Biologicals),

35   PAI-1 (BD Biosciences Pharmingen, United States Biological), angiopoetin-2 (Chemicon, Zymed), PIGF (Cell Sciences), HLA-G (Novus Biologicals, Serotec Inc.), HB-EGF (R&D Systems), TGFβ-3 (Genex Bioscience), decorin (United States Biological).

Diagnostic tools for the measurement of blood pressure or protein content of the urine are known to the person skilled in the art. For example, the protein content of the urine may be measured by dip sticks.

5

In another preferred embodiment, a nucleic acid as defined above, a ligand as defined above, or, additionally, a diagnostic agent as defined above is present on an array.

Said array contains at least two nucleic acids as defined above, or ligands as defined above, or, additionally, diagnostic agents.

10

According to the present invention, the term "array" refers to a solid-phase or gel-like carrier upon which at least two compounds are attached or bound in one-, two- or three-dimensional arrangement. Such arrays are generally known to the person skilled in the art and typically generated on glass microscope slides, specially coated glass slides such as polycation-, nitrocellulose- or biotin-coated slides, cover slips, and membranes such as, for example, membranes based on nitrocellulose or nylon.

15

The aforementioned array may include a bound ligand, an above-defined nucleic acid, or at least two cells expressing at least one ligand or above-defined nucleic acid. In addition, said arrays may include a diagnostic agent or at least two cells expressing such diagnostic agent. Such arrays can be employed for diagnosis of preeclampsia and related syndromes.

20

The invention further relates to a method of producing arrays as defined above, wherein at least one above defined nucleic acid or ligand is bound to the carrier material in addition to other nucleic acids, ligands, or, additionally, diagnostic agents. Preferably, diagnostic agents for at least five, more preferably at least eight, additional marker genes or proteins are additionally bound to the carrier material.

25

Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305). Such arrays can also be brought into contact with substances or substance libraries and tested for interaction, for example for binding or change of confirmation. Therefore, arrays comprising a peptide or polypeptide as defined above may be used for identifying ligands binding specifically to said peptides or polypeptides.

30

35

The invention further relates to a method for preparing an array immobilized on a support material for diagnosis of preeclampsia or a related syndrome, in which at least two nucleic acids, at least two polypeptides or at least two antibodies or antibody fragments, and/or at least two cells or at least one of the aforementioned components in combination with other components relevant to preeclampsia and related disorders are used for preparation. The arrays produced by such a method can be employed for diagnosis of preeclampsia or a related syndrome.

In another preferred embodiment, diagnosis relates to the diagnosis of syndromes related to preeclampsia.

According to the present invention, syndromes "related" to preeclampsia include other pregnancy-associated disorders or syndromes with similar aetiology and/or symptoms as preeclampsia. Particularly included are eclampsia, intrauterine growth retardation, "missed abortion", superimposed gestosis, and HELLP syndrome.

In another embodiment, the present invention also relates to a method of diagnosis in which the above defined nucleic acid molecules, ligands, or, additionally, diagnostic agents or tools are used.

Using the above-defined nucleic acid molecules or the above-defined peptides or polypeptides, it is possible to identify suitable ligands by methods known in the art.

Therefore, the present invention also relates to the use of any of the nucleic acid molecules as defined above or the use of peptides or polypeptides comprising peptides or polypeptides as defined above for the identification of ligands binding specifically to said nucleic acids, peptides or polypeptides.

Suitable methods may comprise the steps of binding ADAM 12 polypeptides or suitable fragments thereof (such as the isoform-specific C-termini) or a candidate for a ligand to a solid phase, contacting the polypeptide with the ligand and detecting the binding of the ligand to the polypeptide. Examples comprise affinity chromatography, yeast-two-hybrid screens and enzyme-linked immuno-sorbent assays (ELISA). Identification of suitable ligands is also offered by commercial suppliers (e.g. Graffinity, Heidelberg), including the identification of binding antibodies, aptamers, and small molecules.

Therefore, the present invention also relates to a method for the identification of ligands binding specifically to any of the peptides or polypeptides as defined above, comprising the steps of contacting the polypeptide with at least one candidate for a ligand, and measuring the binding of the candidate for a ligand to the polypeptide. Said method may
5 further comprise the steps of providing the polypeptide and providing at least one candidate for a ligand. Said peptides or polypeptides may be provided by organic synthesis or recombinant expression using the nucleic acid molecules as defined above. The peptides or polypeptides may also be isolated or purified from body fluids or tissue from the dead or alive human or animal body. Suitable purification methods are well known in the art and
10 include salt fractionation, size exclusion chromatography, ion exchange chromotography, reverse phase chromatography, and affinity chromatography (with or without help of suitable tags, such as His-tags and the like). The isolated or purified peptides or polypeptides may contain modifications, such as glycosylation, against which a suitable ligand or candidate for a ligand may be directed.
15

After contacting the polypeptide with the candidate for a ligand, one or more optional steps of washing can be included.

The method can be carried out in the solid phase or in the liquid phase. If the method is
20 carried out in the solid phase it may comprise a step of binding the polypeptide or a fragment thereof or the test substance to a solid phase. However, the step of contacting the polypeptide with at least one candidate for a ligand may still be carried out in a liquid, particularly in an aqueous solution. Examples for such methods are affinity chromatography and enzyme-linked immuno-sorbent assays (ELISA).
25

In another preferred embodiment, the method may be carried out in the liquid phase. Examples for such methods may include UV-crosslinking, immunoprecipitation, nuclear magnetic resonance spectroscopy (NMR, also available commercially, e.g. by Combinature Biopharm).
30

In another preferred embodiment, a method for identification of ligands may comprise the steps of (a) coupling a peptide or polypeptide comprising a peptide or polypeptide as defined above with a further detection moiety, (b) coupling the candidate for a ligand with a second detection moiety that is capable of interacting with the first detection moiety, (c)
35 contacting the polypeptide of step (a) with the candidate for a ligand of step (b), preferably in a liquid, more preferably an aqueous solution, (d) measuring the interaction of the

detection moieties and thereby detecting the binding of the candidate for a ligand to the polypeptide.

An aqueous solution according to the present invention also relates to a cytosolic solution.
5   Examples for such methods include fluorescent resonance energy transfer (FRET) and yeast-two-hybrid screens. The interaction between the detection moieties may be either covalent or non-covalent, preferably non-covalent. Examples for suitable detection moieties include fluorescent molecules, such as green fluorescent protein (GFP) and its derivatives (Miyawaki, A., Tsien, R.Y., Monitoring protein conformations and interactions
10   by fluorescence resonance energy transfer between mutants of green fluorescent protein. Methods Enzymol. 2000; vol. 327, pp. 472-500) and transcription factors such as Gal4 and VP-16. Another optional step for a method of identification of ligands may comprise the step of comparing the binding of the candidate for a ligand to a related polypeptide, i.e. to another ADAM, such as ADAM 19, ADAM 33, or ADAM 2, to determine the specificity
15   of binding.

In another embodiment, the present invention relates to a method for diagnosis of preeclampsia or a related syndrome comprising the steps of (a) bringing a tissue sample or body fluid sample in contact with a nucleic acid as defined above or a specifically binding
20   ligand as defined above, and (b) detecting the binding of the nucleic acid or ligand. Preferably, the method is carried out in vitro.

Optionally, the method may include a step of obtaining a tissue sample or body fluid sample. Another optional step includes isolating nucleic acids or proteins contained in the
25   sample.

In another embodiment, the present invention relates to the use of a nucleic acid as defined above, a ligand as defined above, optionally in combination with a diagnostic agent or diagnostic tool as defined above, for the manufacture of a diagnostic for the diagnosis of
30   preeclampsia or a related syndrome.

In another embodiment, the present invention relates to a diagnostic containing a nucleic acid molecule, ligand, or, additionally, diagnostic agent as defined above. Such a diagnostic may be used for the diagnosis of preeclampsia or a related syndrome. The
35   nucleic acid molecule, ligand, or, additionally, diagnostic agent in the diagnostic may be labeled or unlabeled. The nucleic acid, ligand or diagnostic agent may also be present on a suitable carrier, e.g. a dipstick.

- 24 -

In another embodiment, the present invention relates to a diagnostic kit containing a nucleic acid molecule, ligand, or, additionally, diagnostic agent and/or diagnostic tool as defined above. Such a diagnostic kit may be used for the diagnosis of preeclampsia or a related syndrome.

The nucleic acid molecule, ligand, or, additionally, diagnostic agent in the kit may be labeled or unlabeled. Additionally, the kit may contain specifically binding secondary or higher order ligands (e.g. antibodies) and auxiliary reagents and tools as appropriate and known in the art. Furthermore, the kit may contain control reagents, such as agents for measuring total RNA, total protein content, or the expression level of "housekeeping" genes or proteins, such as EF-1α, β-tubulin, cyclophilin B, HPRT, GAPDH, and others. The assays to be performed with the kit may be liquid phase assays as well as solid phase assays (i.e. with one or more reagents immobilized on a support). The kit may also comprise suitable tools for obtaining tissue or body fluid samples, particularly blood samples.

In another embodiment, the present invention also relates to a diagnostic or a diagnostic kit, wherein said nucleic acid, ligand or, additionally, diagnostic agent is present on an array. Preferably, diagnostic agents for at least five, more preferably at least eight, additional marker genes or proteins are additionally bound to the carrier material.

In another aspect, the invention relates to a method for the preparation of a diagnostic or a diagnostic kit, wherein a ligand of the peptides or polypeptides as defined above is identified according to the methods as defined above, synthesized in adequate amounts, and formulated into the diagnostic or diagnostic kit.

Similarly, said ligand may provided by any of the methods described above and formulated into the diagnostic or diagnostic kit.

The term "adequate amounts" of the ligand relates to any amounts sufficient for detection and/or measurement of said peptides or polypeptides. Suitable amounts depend on the particular method of detection or measurement, and are known to the person skilled in the art.

With respect to treatment, the problem according to the present invention is solved by use of a nucleic acid molecule comprising a nucleic acid selected from the group consisting of

a) a nucleic acid with a sequence as presented in SEQ ID NO: 1 or 3;

b) a fragment of any of the nucleic acids as defined in a);

c) a nucleic acid with the antisense sequence of any of the sequences defined in a), or b);

d) single-stranded or double-stranded RNA with a sequence corresponding to any of the sequences defined in a), b), or c);

e) siRNA with a sequence defined in a), b), c), or d)

for the manufacture of a medicament for the treatment of preeclampsia or a related syndrome.

The problem is also solved by use of an inhibitor of the biological activity of a peptide or polypeptide with a sequence selected from the group consisting of

a) an amino acid sequence as presented in SEQ ID No. 2 or 4;

b) an amino acid sequence exhibiting a sequence identity with any of the sequences according to a) of at least 85% over 100 residues;

c) a fragment of any of the sequences as defined above

for the manufacture of a medicament for the treatment of preeclampsia or a related syndrome.

These nucleic acid molecules, nucleic acids, peptides, polypeptides and sequences are in the following included in the group of "above defined" nucleic acid molecules, nucleic acids, peptides, polypeptides or sequences.

Preferably, the nucleic acid molecules, nucleic acids, peptides, or polypeptides have been purified and/or isolated.

As a consequence of the present invention, it is known that upregulation of ADAM 12, particularly ADAM 12-S, plays a significant role in preeclampsia and related syndromes. Therefore, treatment by inhibiting expression or biological activity of ADAM 12, particularly ADAM 12-S, is an object of the present invention.

A particular advantage of the present invention is that inhibition does not have to be complete. Already a reduction of the biological activity of ADAM 12, preferably to near

normal levels, can be sufficient for treatment. Thus, inhibition is also understood as a reduction of the biological activity of ADAM 12. Inhibition may also relate to modulation of ADAM 12 biological activity, such as modifying its substrate binding specificity or protease activity. It will be appreciated that inhibition of the biological activity can also be

5   exerted through inhibition of expression, including inhibition of transcription, translation, or a decrease of transcript or protein stability.

According to the present invention, the term "inhibitor" relates to any substance inhibiting the biological activity of ADAM 12 or ADAM 12-S, particularly inhibiting the proteolytic

10   or metalloproteolytic activity of ADAM 12 or ADAM 12-S. It also relates to inhibiting the binding of ADAM 12 or ADAM 12-S to endogenous substrates, such as HB-EGF or IGFBPs. It will be appreciated that all substrates of the proteolytic activity of ADAM 12 may be used as competitive inhibitors, including suitable peptides derived from those substrates. The person skilled in the art is familiar with identifying such peptides.

15

Preferably, the medicament is an inhibitor of expression or biological activity of ADAM 12 or ADAM 12-S. Inhibition of expression includes inhibition of transcription, translation, or a decrease of transcript or protein stability. Inhibition of biological activity particularly relates to inhibition of substrate binding activity or proteolytic activity.

20

A medicament according to the present invention preferably comprises additionally a suitable pharmaceutically acceptable carrier. The medicament may additionally comprise other carrier substances, preferably starch, lactose, fats, stearin acid, alcohol, physiological NaCl-solutions or further additives, in particular stabilizers, preservatives, dyes and

25   flavourings. The medicament may also comprise other suitable substances. For example, an RNA or siRNA containing medicament may contain substances which stabilize RNA, particularly double-stranded RNA.

The above defined nucleic acid molecules are preferably used to inhibit expression of the

30   corresponding endogenous genes. Nucleic acids which can be used to inhibit expression of the endogenous genes are known to the person skilled in the art. For example, peptide nucleic acids, comprising sequences as defined above, can suppress expression of the corresponding endogenous gene by forming DNA triplex structures with the gene. Other nucleic acids, such as antisense RNAs, siRNAs, morpholino oligonucleotides, or

35   ribozymes, can be used to interfere with RNA transcribed from the endogenous gene. In

particular, small interfering RNAs (siRNA) can be used for inhibition of expression of the endogenous gene.

In a preferred embodiment, the nucleic acids have sequences shown in SEQ ID NO: 1 or 3,
5   which correspond to those presented in GenBank Acc. No. AF023476 or AF023477.

In another preferred embodiment, fragments of any of the nucleic acids as defined above are used, preferably fragments with sequences located in SEQ ID NO: 5 or SEQ ID NO: 7. Suitable fragments are known to the person skilled in the art. For example, suitable
10   fragments may be a peptide nucleic acid or oligonucleotides or nucleic acid fragments encoding siRNAs (also described in Tuschl, Nature Biotechnology, Vol. 20, pp. 446-44).

In another preferred embodiment, said nucleic acids have the antisense sequence of any of the sequences defined above. Such nucleic acids may comprise peptide nucleic acids
15   (PNAs), antisense morpholino oligonucleotides, or antisense or double-stranded RNAs. Therefore, in another preferred embodiment, said nucleic acids may be single-stranded or double-stranded RNA with sequences corresponding to any of the sequences defined above.

20   In another preferred embodiment, the above-defined nucleic acid is an siRNA with sequences as defined above.

The inhibition of a specific target gene in mammals is achieved by the introduction of an siRNA-molecule having a sequence that is specific for the target gene into the mammalian
25   cell. The siRNAs comprise a first and a second RNA strand, both hybridized to each other, wherein the sequence of the first RNA strand is an oligonucleotide corresponding to one of the sequences as defined above for therapy and wherein the sequence of the second RNA strand is the antisense-strand of the first RNA strand. The siRNA-molecules may possess a characteristic 2- or 3-nucleotide 3'-overhang. Each strand of the siRNA molecule
30   preferably has a length of 19 to 31 nucleotides.

The siRNAs can be introduced into the mammalian cell by any suitable known method of cell transfection, particularly lipofection, electroporation, or microinjection, be it in vitro or in vivo.

35

The RNA oligonucleotides can be generated and hybridized to each other in vitro or in vivo according to any of the known RNA synthesis methods, in order to obtain siRNA.

5   In another embodiment, the invention relates to the use of a nucleic acid molecule, wherein the nucleic acid molecule is contained in at least one nucleic acid expression vector which is capable of producing a double-stranded RNA-molecule comprising a sense-RNA-strand and an antisense-RNA-strand under suitable conditions, wherein each RNA-strand, independently from the other, has a length of 19 to 31 nucleotides.

10   In this alternative method (also described in Tuschl, Nature Biotechnology, Vol. 20, pp. 446-448), vector systems capable of producing siRNAs instead of the siRNAs themselves are introduced into the mammalian cell for downregulating gene expression.

In a preferred embodiment, the first and the second RNA strand of the siRNA may have,
15   independently from the other, a length of 19 to 25 nucleotides, more preferred of 20 to 25 nucleotides, and most preferred of 20 to 22 nucleotides.

In another preferred embodiment, the first and the second RNA strand of the siRNA may have, independently from the other, a length of 26 to 30 nucleotides, more preferred of 26
20   to 28 nucleotides, and most preferred of 27 nucleotides.

In another embodiment, the present invention relates to a medicament comprising an above-defined nucleic acid molecule.

25   Administration of the medicament can be carried out by known methods. For therapeutic applications, the medicament may be in form of a solution, in particular an injectable solution, a cream, ointment, tablet, suspension, granulate or the like. The medicament may be administered in any suitable way, in particular by injection, by oral, nasal, rectal application. The medicament may particularly be administered parenteral, that means
30   without entering the digestion apparatus, for example by subcutaneous injection. In particular, the medicament may also be injected intravenously in the form of solutions for infusions or injections. Other suitable administration forms may be direct administrations on the skin in the form of creams, ointments, sprays and other transdermal therapeutic

substances or in the form of inhalative substances, such as nose sprays, aerosoles or in the form of microcapsules or implantates.

Preferably, the inhibitor is administered in pharmaceutically effective amount. As used
5   herein, a "pharmaceutically effective amount" of an inhibitor is an amount effective to achieve the desired physiological result, either in cells treated *in vitro* or in a subject treated *in vivo*. Specifically, a pharmaceutically effective amount is an amount sufficient to positively influence, for some period of time, one or more clinically defined pathological effects or symptoms associated with preeclampsia. The pharmaceutically effective amount
10   may vary depending on the specific inhibitor selected, and is also dependent on a variety of factors and conditions related to the subject to be treated and the severity of the disorder or symptom. For example, if the inhibitor is to be administered *in vivo*, factors such as age, weight, gestational age, and general health of the patient as well as dose response curves and toxicity data obtained in pre-clinical animal tests would be among the factors to be
15   considered. If the inhibitor is to be contacted with cells *in vitro*, one would also design a variety of pre-clinical *in vitro* studies to asses parameters like uptake, half-life, dose, toxicity etc. The determination of a pharmaceutically effective amount for a given agent is well within the ability of those skilled in the art. Preferably, the inhibitor is present in a concentration of 0,1 to 50% per weight of the pharmaceutical composition, more
20   preferably 10 to 30%.

In another embodiment, the present invention relates to a method of treatment of preeclampsia or a related syndrome comprising the use of the above-identified nucleic acid molecules.
25

Treatment of preeclampsia or a related syndrome relates to treatment of the disorder or syndrome itself as well as treatment of symptoms associated with said disease or syndrome. Such symptoms may include intravascular coagulation, blood platelet destruction, high blood pressure, edema, proteinuria, or placental abruption. Treatment
30   according to the present invention also relates to prophylaxis.

Treatment according to the present invention may be accompanied by treatment approaches known in the art, for example blood pressure control (particarly by

administration of alpha-receptor blockers (e.g. hydralazine) or ebrantil), tocolysis (particularly for increase of placental blood flow), administration of magnesium, and administration of cortisone.

5   In another preferred embodiment, the inhibitor is a disintegrin domain metalloproteinase inhibitor, particularly KB-R7785, a TIMP, particularly TIMP-3 or a fragment thereof, α_2-macroglobulin, or an antibody directed against ADAM 12.

In a preferred embodiment, such an inhibiting antibody is directed against the proteolytic
10   domain of ADAM 12, particularly the zink binding domain, more particularly including amino acid positions 350-361.

Preferably, the inhibitor should be specific for ADAM 12, ADAM 12-L, or ADAM 12-S. The criteria for specificity as defined above for ligands apply analogously.
15

In a preferred embodiment, the peptide or polypeptide is a fragment with a sequence consisting of or located in SEQ ID NO: 8. According to this embodiment, the inhibitor is directed specifically against a peptide or polypeptide consisting of or located in SEQ ID NO: 8. An example is an antibody raised against such a peptide or polypeptide. It is known
20   that such antibodies, though in this case binding only to the C-terminus of ADAM 12-S, can sterically inhibit the biological activity of the entire ADAM 12-S protein. The same is true for aptamers and other inhibitors. Suitable inhibitors can be found by identifying ligands binding specifically to ADAM 12-S and subsequently screening them as candidates for inhibitors. Analogously, inhibitors directed against the C-terminus of ADAM 12-L
25   (SEQ ID NO: 6) may be identified.

In another preferred embodiment, the above-defined nucleic acid or inhibitor is used in conjunction with HB-EGF. Preferably, HB-EGF has been purified or isolated. Preferably, HB-EGF is formulated into the medicament defined above.
30

In another preferred embodiment, the medicament is for treatment of symptoms of preeclampsia or a related syndrome, particularly for treatment or prevention of intravascular coagulation, blood platelet destruction, high blood pressure, edema, glomerular endotheliosis, proteinuria, or placental abruption.
35

Symptoms of preeclampsia and related syndromes include any undesired effects the disorders may have on the mother or fetus. Particularly, symptoms relate to symptoms

caused by a high level of ADAM 12 in the blood, particularly the maternal blood. More particularly, such symptoms include intravascular coagulation (thrombosis), blood platelet destruction, or high blood pressure (particularly above 140/90 mm Hg), edema (swelling of face and/or hands), glomerular endotheliosis, or proteinuria (particularly more than 0.3 g/24 hours or a level of 2+ by dipstick test).

Treatment may also be restricted to the mother's blood. For example, IgM antibodies or other molecules of high molecular weight have only a limited capability of passing the placental blood barrier. Therefore, in a preferred embodiment, the inhibitor may be an IgM or any other molecule not capable of passing the placental blood barrier, particularly being of similar or higher molecular weight as an IgM. For example, an identified inhibitor, such as an aptamer, may be coupled covalently or non-covalently to a high molecular weight residue, such as a polypeptide or dextran.

In another aspect, the present invention relates to the use of the nucleic acids, peptides or polypeptides as defined above for identification of inhibitors of the biological activity of the above-defined nucleic acids, peptides or polypeptides, particularly for identification of inhibitors of the proteolytic activity or substrate-binding activity of such peptides or polypeptides.

The person skilled in the art is familiar with such use of nucleic acids, peptides or polypeptides. Examples for suitable methods are described in the following.

Therefore, in another aspect, the present invention relates to a method for identification of inhibitors of any of the peptides or polypeptides as defined above, comprising the steps of (a) contacting said peptide or polypeptide with a suitable substrate, e.g. HB-EGF, (b) measuring the decrease in processing of the substrate in the presence as compared to the absence of a candidate for an inhibitor molecule.

Suitable substrates include all substrates of biological, preferably enzymatic, more preferably proteolytic activity. Suitable substrates include HB-EGF and IGFBPs, particularly IGFBP-3 and IGFBP-5. Processing of the substrate relates preferably to enzymatic modification, more preferably proteolytic modification or cleavage of the substrate. Measurement of processing can be done e.g. by measuring the decrease in substrate (educt) and the increase in modified substrate (product). Measurement of processing can involve indirect measurement, e.g. via ligands, labeled or unlabeled (see above) binding to the substrate or modified substrate.

Candidates for inhibitor molecules can be any nucleic acid, peptide, polypeptide or other substance. In particular, candidates may include peptides derived from TIMP, IGFBP-3, IGFBP-5, HB-EGF, α_2-macroglobulin, antibodies, nucleic acid or protein aptamers or
5 fragments or derivatives of any of these substances. Other candidates for inhibitor molecules may comprise derivatives of KB-R7785. The person skilled in the art is familiar with methods of generating such derivatives, either by methods of combinatorial chemistry or introducing mutations into the respective nucleic acids or polypeptides.

10 In a preferred embodiment, the present invention relates to the candidate for inhibitor being a substrate or ligand as defined above. Any substrate of ADAM 12 or ADAM 12-S or ligand as defined above is also considered a candidate for an inhibitor.

In another aspect, the present invention relates to a method for the preparation of a
15 pharmaceutical composition, wherein an inhibitor of the nucleic acids, peptides or polypeptides as defined above is identified according to the method as defined above, synthesized in adequate amounts, and formulated into a pharmaceutical composition.

Suitable methods to synthesize the inhibitor molecules are known in the art. For example,
20 peptides or polypeptides can be synthesized by recombinant expression (see also above), and antibodies can be obtained from hybridoma cell lines or immunized animals. Small molecules can be synthesized according to any known organic synthesis method.

Similarly, said inhibitor may be provided by any of the screening methods described above
25 and formulated into a pharmaceutical composition.

The term "adequate amounts" of the inhibitor relates to any amounts sufficient for inhibiting expression or biological activity of ADAM-12 in the human or animal body, preferably the human body. In particular, adequate amounts relate to pharmaceutically
30 effective amounts.

Description of the Figures

Fig. 1 Increased ADAM 12-S expression in preeclamptic human placenta. A, gestational
age (GA, weeks + days) of the study subjects (Pat. No.). B, Semi-quantitative RT-
35 PCR analysis for the levels of ADAM 12-S transcripts in human placenta using
cyclophilin B for normalization. Note the increased level of ADAM 12-S transcript
in samples of preeclamptic patients (Preecl.) as compared samples of healthy

mothers (Ctrl.). The increase is visible both in the fetal (f) and maternal (m) part of the placenta. Patient 22 was pregnant with twins, and samples 22-1 and 22-2 were derived from the placentae belonging to each of the twins.

Fig. 2   Increased ADAM 12-S expression in preeclamptic human placenta. Shown are relative mRNA levels for ADAM 12-S in the fetal (f) or maternal (m) part of healthy (Ctrl.) and preeclamptic (Preecl.) human placentas as determined by real time PCR using elongation factor EF-1α for normalization. Note the increased level of mRNA in samples of preeclamptic patients as compared samples of healthy pregnant women. Y-axis: level of fluorescence of the PCR product (which is correlated with the level of mRNA in the sample), X-axis: Sample numbers, corresponding to the patient numbers (Pat No.) in Fig. 1A. Patient 22 was pregnant with twins, and samples 22-1 and 22-2 were derived from each placenta of twin siblings.

The invention is further illustrated by the following examples, which are not intended to limit the scope of the invention in any way.

EXAMPLE 1

Placentae from different gestational age from preeclamptic patients as well as from healthy control patients were dissected and collected within less than 15 min after delivery. Preeclampsia was diagnosed according to the generally accepted criteria (blood pressure above 140/90 mm Hg and proteinuria of more than 0.3 g/24 hours or a level of 2+ by dipstick test). 10-20 mm thick sections were dissected and the fetal and maternal side were collected separately. Tissue was either immediately snap frozen or fixed in 4% paraformaldehyde for 12 hours. The fixed tissue was washed twice in phosphate-buffered saline (PBS, composition of 1l: 8g NaCl (137 mM), 0,2g KCl (2,7 mM), 1.44g $Na_2HPO_4 \cdot 2H_2O$ (8,1 mM), 0,24g KH_2PO_4 (1,76mM), pH 7.4), transferred to 70% ethanol and paraffin embedded. For RNA preparation the tissue was incubated for 24 hrs in RNALater (Ambion) at 4°C and then frozen at -80°C.

EXAMPLE 2

Total RNA was isolated from frozen placental tissue, either from healthy or preeclamptic pregnant women of different gestational age, with Trizol (Invitrogen, Karlsruhe, Gemany) according to the manufacturer's recommendations. The integrity and purity of the total RNA was checked on a 1% agarose gel with denaturing loading buffer or on the RNA 6000 Nano Chip (Agilent Technology, Germany). Total RNA quality and concentration

was determined by absorption spectroscopy between 220 and 320 nm (1 OD^{260} = 40 μg/ml ssRNA).

EXAMPLE 3

5   The microarrays contained 1600 nonredundant, sequence-validated human cDNAs (RZPD set, Berlin, (Fritz, B., et al. (2002). Microarray-based copy number and expression profiling in dedifferentiated and pleomorphic liposarcoma. Cancer Res, vol. 62: 2993-8). The cDNAs had been selected on their putative involvement in tumorigenesis and were PCR amplified as described previously (Wrobel, G., et al. (2003). Optimization of high-
10   density cDNA-microarray protocols by 'design of experiments'. Nucleic Acids Res, vol. 31: e67, and Schlingemann, J., et al. (2003). Profile of gene expression induced by the tumor promotor TPA in murine epithelial cells. Int J Cancer 104(6), p. 699-708), purified by isopropanol precipitation and dissolved in 20 μl FBNC (Formamid, betaine and nitrocellulose) spotting buffer (Wrobel, G., et al. (2003), see above). The resulting cDNA
15   fragments had an average length between 500-1500 bp.

EXAMPLE 4

DNA spotting was performed on Poly-Prep poly-L-lysine coated substrates (Sigma Chemical Co.; However, also other substrates known in the art may be used, such as QF
20   Epoxy substrates (Quantifoil Microtools, Jena, Germany)) using an OmniGrid Microarrayer (GeneMachines, San Carlos, CA) equipped with Stealth SMP3 Micro Spotting Pins (Telechem). Spot centres were 250 μm apart. The arrays were provided with positive control spots of the Spot Report System (Stratagene, Amsterdam, The Netherlands) as well as a variety of additional negative controls, e.g., mouse Cot-1 DNA
25   (Invitrogen), yeast tRNA (Sigma Chemical Co.) and poly-dA (Amersham Biosciences). Furthermore, Cy3- and Cy5-labeled DNA was included to yield specific fluorescent landmarks on the arrays. DNA immobilisation to the glass was achieved by 3.5 hours of incubation at 80°C, followed by 2x irradiation at 254 nm with an energy of 120 mJ/cm² in a Stratalinker Model 2400 UV illuminator (Stratagene).

30

EXAMPLE 5

30-40 μg of total RNA (as obtained according to Example 2) was used for microarray hybridisation. Reverse transcription was performed in accordance to Wrobel et al., 2003 using an Omniscript RT Kit (QIAGEN) and C3/Cy5-dUTPs (NEN Life Science Products,
35   Köln, Germany). Subsequently, Cy3- and Cy5-labeled cDNA samples were pooled (age matched placental tissue from healthy and preeclamptic placental tissues), purified and

concentrated to 10 μl using a Microcon YM-30 PCR filter unit (Millipore, Eschborn, Germany), as previously described.

EXAMPLE 6

5   31 μl of DIG Easy Hybridisation buffer (Roche Diagnostics, Mannheim, Germany) were added to each cDNA pool and incubated for 15-60 min at room temperature. Denaturation was carried out at 65°C for 3 min. Hybridisation was performed for 16h at 37°C in ArrayIt Hybridisation cassettes (TeleChem International, Sunnyvale) in a waterbath. Thereafter the slides were washed at room temperature with (i) 1x SSC, 0.1% (w/v) SDS for 10 min for 3

10   times; (ii) 0.1x SSC, 0.1% SDS for 10 min; (iii) H_2O for 3 min; (iv) 70% ethanol for 3 min; (v) 90% ethanol for 3 min; (vi) 100% ethanol for 3 min and (vii) finally dried with compressed air.

EXAMPLE 7

15   Microarrays were scanned and analysed using a GenePix 4000A microarray scanner (Axon Instruments, Inc., Union City, NJ), controlled by the software GenePix Pro 3.0 (Axon Instruments, Inc.). Individual data reports for all conducted experiments (including, e.g., median fluorescence intensities of all spots and local background) were exported and further analysed using the R software environment for statistical computing (http://www.r-

20   project.org) as well as Microsoft Excel 2000. Here, data sets for spots not recognized by the GenePix analysis Software were excluded from further considerations. Additionally, all remaining data sets were ranked according to spot homogeneity (as assayed by the ratio of median and mean fluorescence intensities), spot intensity and the standard deviation of log ratios for replicate spots. Those data points ranked among the lower 50%, and thus

25   possessing the lower reliability based on the criteria just described, were eliminated. Data sets that could not be verified, either in a colour switch experiment (reversed assignment of fluorophores) or on a different experimental day, were omitted as well. For each hybridisation, fluorescence ratios (Cy5/Cy3) were normalised by the median log ratio of all genes found in this experiment. Accurate differential expression values for each gene were

30   obtained by calculating the average of the normalised ratios of all independent hybridisations, successfully identifying that gene, and reversing the ratios from colour switch experiments prior to this operation.

EXAMPLE 8

35   Comparative microarray analyses were performed for with the microarray as described in Example 3. The following pairs of samples with the indicated gestational ages (gestational weeks + days) were used:

Pair No.	healthy (control)	preeclamptic
1	41+3	41+0
2	38+2	38+2
3	34+0	35+1 (1)
4	34+0	35+1 (2)
5	34+0	33+2

35+1 (1) and 35+1 (2) designate samples derived from each placenta of twin siblings (patient 22, see legend to Fig. 1 and 2).

It was found that ADAM-12 was the most strongly regulated gene. Shown below are the data of Pair No. 2. Expression levels were calculated from two independent experiments.

	induced (upregulated)			repressed (downregulated)		
Part of placenta	fetal	maternal	both	fetal	maternal	both
No. of genes induced more than 2-fold	35	27	27	35	35	34
ADAM-12	38-fold	44.5-fold		-	-	
TIMP-1	8.5-fold	12-fold		-	-	
TIMP-2	13-fold	11.5-fold		-	-	
EPAS/HIF-2α	4-fold	2.5-fold		-	-	
IGFBP-1	-	-		less than 2-fold	7.5-fold	
IGFBP-3	-	-		2.6-fold	5.7-fold	
TEK	-	-		4.8-fold	2.8-fold	
decorin	-	-		less than 2-fold	6.5-fold	

ADAM 6 and ADAM 9 were also included on the microarray, but were not found to be induced or repressed by more than 2-fold.

EXAMPLE 9

For RT-PCR analysis 2-5 μg total RNA from healthy and preeclamptic placental tissue of different gestational age pretreated with RQ1-DNase (Promega, Madison, WI) were reverse transcribed in a reaction volume of 20 μl using AMV-RT (Promega) and an oligo-d(T) (Promega) according to the manufacturer's recommendations, 2-3 μl of the RT reaction together with primers depicted in the following table were used for PCR detection.

Primer Pairs used for RT-PCR analysis		
Gene Name	Forward primer 5´	Reverse primer 3´
ADAM 12-L	CCTGTGACCTCCCAGAGTTCTGC (SEQ ID NO: 9)	GAATTCCTATGGTTAAACCTTGG (SEQ ID NO: 10)
ADAM 12-L	CTTGCTGCCGGATTTGTGGTTTAT (SEQ ID NO: 11)	AGTTGACTGGGGCTGAGGGACATT (SEQ ID NO: 12)
ADAM 12-S	CCTGTGACCTCCCAGAGTTCTGC (SEQ ID NO: 9)	GGACTCTGCAGCTTCCTGCCTTG (SEQ ID NO: 14)
ADAM 12-S	TCGGGGCTCAGGAGGGGAAGT (SEQ ID NO: 15)	GGCTGCCAAGGCGGAGGTGTC (SEQ ID NO: 16)
human cyclophilinB	GGCCGGGTGATCTTTGGTCTCTTC (SEQ ID NO: 17)	CCCGGCTGTCTGTCTTGGTGCTCT (SEQ ID NO: 18)
human EF-1α	CTGCTGAGATGGGAAAGGGCT (SEQ ID NO: 19)	TTCAGGATAATCACCTGAGCA (SEQ ID NO: 20)

Human cyclophilin B served as an internal control for the quality and quantity of the cDNA. Amplified probes were separated on 1.5% agarose gels in the presence of ethidium bromide and visualised by using the Eagle Eye system (Stratagene). To obtain a calibration graph, cDNA PCR product from human elongation factor EF-1α was serially diluted in H_2O (five 10-fold dilutions from 0.01 pg to 10 pg). To standardize the amount of sample cDNA, an endogenous control amplicon was used (EF-1α).

EXAMPLE 10

Semi-quantitative RT-PCR was performed on placental tissue samples obtained from 4 healthy and 3 preeclamptic patients (Fig. 1A). Typical results of the RT-PCR

amplifications are shown in Fig. 1B. Samples 22-1 and 22-2 were obtained from the same patient.

A comparison of data from healthy and preeclamptic women is shown in the graph of Fig. 2. Overall, the RT-PCR experiments demonstrate a 4 to 5-fold increase in ADAM 12-S transcript in placental tissue of both maternal and fetal origin.

EXAMPLE 11

The following procedure was used to obtain RNA from plasma samples. Peripheral blood samples were collected with informed consent of the patients. 5 to 10 ml blood samples were collected in EDTA-containing tubes and centrifuged at 4000 rpm for 15 min at 4°C. Plasma was collected in new tubes. The plasma samples were recentrifuged at 13000 rpm for 15 min at 4°C.

For analysis of RNA derived from plasma, the supernatants obtained in the above procedure are transferred to fresh tubes and can be processed according to a previously reported protocol, which is herewith incorporated by reference (Ng, E.K.O., Leung, T.N., et al. (2003). The concentration of circulating Corticotropin-releasing hormone RNA in maternal plasma is increased in preeclampsia. Clin. Chem. 49, 727-731, see particularly p. 728-729 and Ng, E.K.O., Tsui, N.B.Y., et al. (2003). Messenger RNA of placental origin is readily detectable in maternal plasma. Proc Natl Acad Sci USA, vol. 100(8):4748-53, see particularly p. 4748-4749).

EXAMPLE 12

For Real-time Quantitative PCR (RQ PCR), the QuantiTect™ SYBR Green PCR Kit (Qiagen, Hilden, Germany) was used to amplify ADAM-12S specific templates from cDNAs of placental RNA of different patients according to the manufacturer's instructions. The amount of the amplified templates in each sample was analysed with the MJ Research Opticon™ (Biozym, Oldendorf, Germany) calculating against a standard PCR with elongation factor EF-1α, amplified from different dilutions.

Quantitative assessment of the DNA amplification was detected via the dye SYBR Green, the fluorescence of which is strongly enhanced upon the binding to the dsDNA. The RQ-PCR reactions were carried out in 50 µl using 1 µl of the RT reaction at a primer concentration of 0.3 µm, respectively, according to the manufacturer's recommandations for QuantiTect™ SYBR Green PCR Kit reagents (Qiagen, Hilden, Germany). The oligonucleotides used for real time PCR are listed in example 9. For thermal cycling the

following conditions were applied: one step at 94°C 15 min, then 40 cycles of 30 sec at 94°C, 1 min at 56°C and 1:30 min at 72°C and a final step at 72°C for 3 min. Melting curve was achieved from 50 up to 93°C within reading every 0.5°C hold for 10 sec between reads to obtain melting curves of the final RQ-PCR products. This is necessary because SYBR Green fluorescence may also be derived from side products such as primer dimers.

To obtain a calibration graph, cDNA PCR product from elongation factor EF-1α was serially diluted in H_2O (five 10-fold dilutions from 0.01 pg to 10 pg). To standardize the amount of sample cDNA, an endogenous control amplicon was used (EF-1α).

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Universität Heidelberg

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Patent Claims

1. Use of a nucleic acid molecule comprising a nucleic acid selected from the group consisting of

    a) a nucleic acid with a sequence as presented in SEQ ID NO: 1 or 3;

    b) a nucleic acid with a sequence that exhibits a sequence identity with any of the sequences defined in a) of at least 70% over 300 residues;

    c) a nucleic acid which is capable of hybridizing with the nucleic acid as defined in a), or b) under conditions of medium or high stringency;

    d) a nucleic acid with the antisense-sequence of any of the sequences defined in a), b) or c);

    e) a fragment of any of the nucleic acids as defined in a), b), c), or d);

    f) an RNA corresponding to any of the sequences defined in a), b), c), d) or e);

    for diagnosis of preeclampsia or a related syndrome.

2. The use according to claim 1, wherein the fragment has a sequence consisting of or being part of SEQ ID NO: 7, 13, 14, 15, or 16, or wherein the fragment has a sequence consisting of or being part of SEQ ID NO: 5, 10, 11, or 12.

3. Use of a ligand binding specifically to a peptide or polypeptide with a sequence selected from the group consisting of

    a) an amino acid sequence as presented in SEQ ID NO: 2 or 4;

    b) an amino acid sequence exhibiting a sequence identity with any of the amino acid sequences according to a) of at least 85% over 100 amino acid residues;

    c) a fragment of any of the sequences defined above

    for diagnosis of preeclampsia or a related syndrome.

4. The use according to claim 3, wherein the sequence is selected from SEQ ID NO: 8.

5. The use according to any of claims 3 to 4, wherein the ligand is selected from the group consisting of

    a) KB-R7785 or a derivative thereof;

    b) TIMP-1, TIMP-2, TIMP-3, IGFBP-3, IGFBP-5, HB-EGF, α_2-macroglobulin, PKC-δ, α-actinin, α-actinin-2, src, Grb-2, or syndecan-4

    c) antibodies;

    d) nucleic acid or protein aptamers;

    e) fragments or derivatives of any of the substances defined in b), c), or d).

6. The use according to any of claims 1 to 5, wherein the nucleic acid or ligand is used in conjunction with a diagnostic agent for the measurement of expression of any of the genes or proteins selected from the group consisting of

    a) EPAS-1/HIF-2α,

    b) neurokinin B

    c) TIMP-1,

    d) VEGFR-1,

    e) VEGF,

    f) IGFBP-1

    g) IGFBP-3,

    h) matrix metalloproteinase-2,

    i) leptin,

    j) PAI-1,

    k) IGF-1,

    l) angiopoetin-2,

    m) decorin,

    n) PIGF,

    o) HLA-G

    p) HB-EGF

    q) TGF-β3

and/or diagnostic tools for the measurement of blood pressure or protein content of the urine.

7. The use according to any of claims 1 to 6, wherein the nucleic acid, ligand or, additionally, diagnostic agent is present on an array.

8. Use of the nucleic acid molecule as defined in any of claims 1 to 2, or of a peptide or polypeptide comprising a peptide or polypeptide as defined in any of claims 3 or 4 for the identification of ligands binding specifically to said nucleic acid, peptide or polypeptide.

9. A method for the identification of ligands binding specifically to a peptide or polypeptide as defined in in any of claims 3 to 4, comprising the following steps:

    a) contacting the polypeptide with at least one candidate for a ligand;
    b) measuring the binding of the candidate for a ligand to the polypeptide.

10. A method for diagnosis of preeclampsia or a related syndrome comprising the following steps:

    a) bringing a biopsy or bodily fluid sample into contact with a nucleic acid as defined in any of claims 1 to 2 or a specifically binding ligand as defined in claims 3 to 5, and
    b) detecting the binding of the nucleic acid or ligand.

11. Use of a nucleic acid as defined in any of claims 1 to 2, or a ligand as defined in any of claims 3 to 5, optionally in combination with a diagnostic agent or diagnostic tool as defined in claim 6, for the manufacture of a diagnostic for the diagnosis of preeclampsia or a related syndrome.

12. A diagnostic containing a nucleic acid or ligand, or, additionally, diagnostic agent or tool as defined in any of claims 1 to 5.

13. A diagnostic kit containing a nucleic acids or ligand as defined in any of claims 1 to 5.

14. The diagnostic or diagnostic kit according to any of claims 12 to 13, wherein the nucleic acid, ligand or, additionally, diagnostic agent is present on an array.

15. Use of a nucleic acid molecule comprising a nucleic acid selected from the group consisting of

    a) a nucleic acid with a sequence as presented in SEQ ID NO: 1 or 3;

5

    b) a fragment of any of the nucleic acids as defined in a);

    c) a nucleic acid with the antisense sequence of any of the sequences defined in a), or b);

    d) single-stranded or double-stranded RNA, preferably siRNA, with a sequence corresponding to any of the sequences defined in a), b), or c);

10

for the manufacture of a medicament for the treatment of preeclampsia or a related syndrome.

16. The use according to claim 15, wherein the fragment consists of or is located in SEQ

15   ID NO: 5 or 7.

17. Use of an inhibitor of the biological activity of a peptide or polypeptide with a sequence selected from the group consisting of

20

    a)   an amino acid sequence as presented in SEQ ID No. 2 or 4;

    b)   an amino acid sequence exhibiting a sequence identity with any of the sequences according to a) of at least 85% over 100 residues;

    c)   a fragment of any of the sequences as defined above

25

for the manufacture of a medicament for the treatment of preeclampsia or a related syndrome.

18. The use according to claim 17, wherein the fragment has a sequence consisting of or being part of SEQ ID NO: 8.

30

19. The use according to any of claims 17 to 18, wherein the inhibitor is a disintegrin domain metalloproteinase inhibitor, particularly KB-R7785, a TIMP, particularly TIMP-3 or a fragment thereof, α_2-Macroglobulin, or an antibody directed against ADAM 12.

35

20. The use according to any of claims 17 to 19, wherein additionally HB-EGF is used for the manufacture of the medicament.

21. The use according to any of claims 15 to 20, wherein the medicament is for treatment of symptoms of preeclampsia or a related syndrome, particularly for treatment or prevention of intravascular coagulation, blood platelet destruction, placental abruption, or high blood pressure.

22. Use of a nucleic acid, peptide, or polypeptide as defined in any of claims 1 to 4 for identification of an inhibitor of said nucleic acid, peptide or polypeptide, particularly for identification of an inhibitor of the proteolytic activity or substrate-binding acitivity of said peptide or polypeptide.

23. A method for identification of an inhibitor of the biological activity of a peptide or polypeptide as defined in any of claims 17 to 18, comprising the following steps:

   a) contacting said peptide or polypeptide with a suitable substrate, e.g. HB-EGF,
   b) measuring the decrease in processing of the substrate in the presence as compared to the absence of a candidate for an inhibitor molecule

24. The method according to claim 22, wherein the candidate for an inhibitor is a substrate or a ligand of a peptide or polypeptide as defined in any of claims 3 to 4.

25. A method for the preparation of a pharmaceutical composition, wherein an inhibitor of the nucleic acids, peptides, or polypeptides as defined in any of claims 17 to 18 is identified according to the methods as defined in claim 22 or 23, synthesized in adequate amounts, and formulated into a pharmaceutical composition.

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Summary

5

The present invention relates to the significant role of ADAM 12, ADAM 12-L, and ADAM 12-S in preeclampsia and related disorders. The invention further relates to the use of said genes and gene products, ligands binding to those, and modulators of activity of the gene products in diagnosis and treatment of preeclampsia and related syndromes.

Fig. 1

A

Pat. No.	GA	Ctrl.	Preecl.
5	38+2	+	-
6	37+2	+	-
20	34	+	-
21	41+3	+	-
14	38+2	-	+
16	33+2	-	+
22	35+1	-	+

B
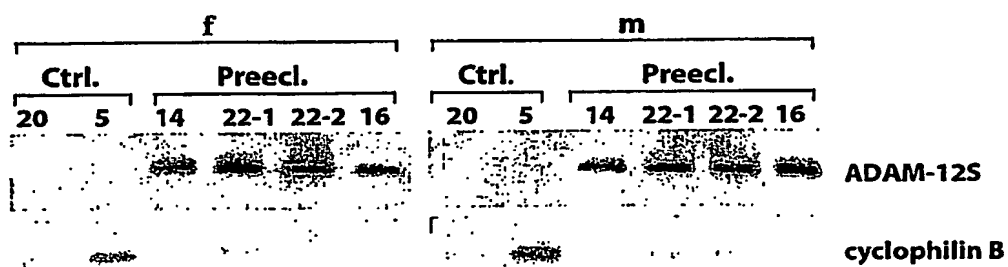
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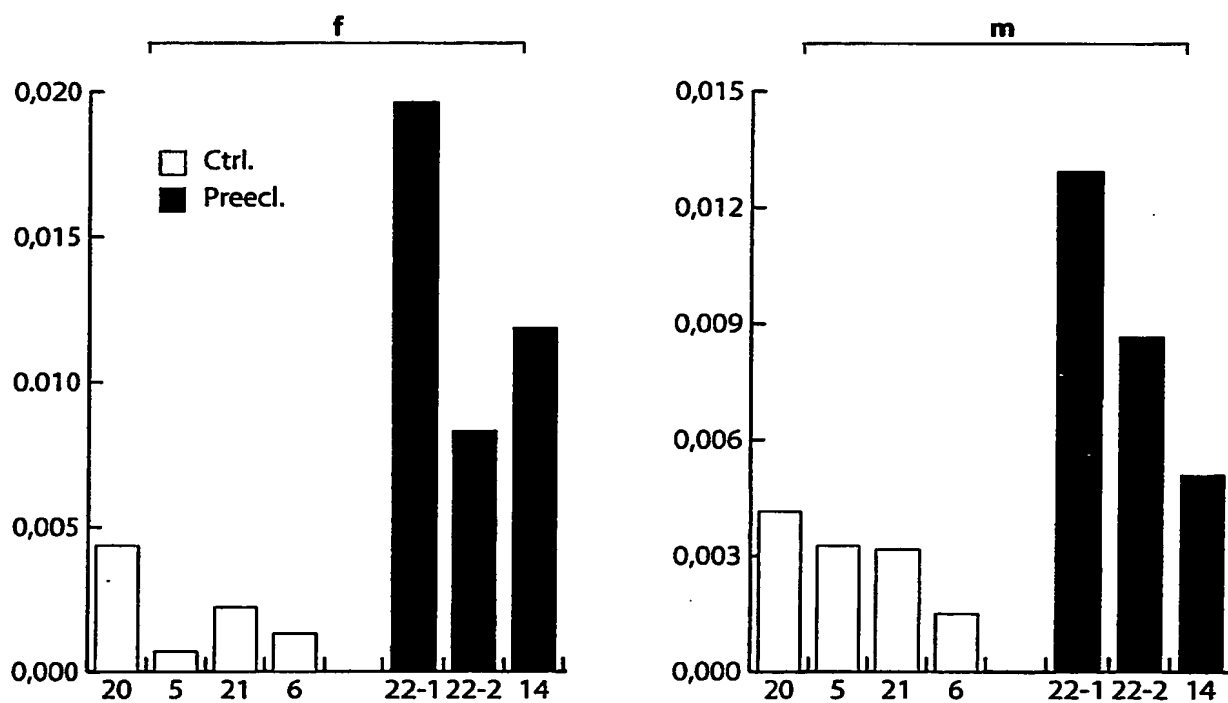
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<120> Use of ADAM 12 for diagnosis and therapy of preeclampsia

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ccggagctga ctcgccgagg caggaaatcc ctccggtcgc gacgcccggc cccgctcggc     240

gcccgcgtgg gatggtgcag cgctcgccgc cgggcccgag agctgctgca ctgaaggccg     300

gcgacg atg gca gcg cgc ccg ctg ccc gtg tcc ccc gcc cgc gcc ctc      348
       Met Ala Ala Arg Pro Leu Pro Val Ser Pro Ala Arg Ala Leu
        1               5                  10

ctg ctc gcc ctg gcc ggt gct ctg ctc gcg ccc tgc gag gcc cga ggg      396
Leu Leu Ala Leu Ala Gly Ala Leu Leu Ala Pro Cys Glu Ala Arg Gly
15                  20                  25                  30

gtg agc tta tgg aac caa gga aga gct gat gaa gtt gtc agt gcc tct      444
Val Ser Leu Trp Asn Gln Gly Arg Ala Asp Glu Val Val Ser Ala Ser
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Val Arg Ser Gly Asp Leu Trp Ile Pro Val Lys Ser Phe Asp Ser Lys
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Leu Ile Ile Asn Leu Glu Arg Asn Glu Gly Leu Ile Ala Ser Ser Phe
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acg gaa acc cac tat ctg caa gac ggt act gat gtc tcc ctc gct cga     636
Thr Glu Thr His Tyr Leu Gln Asp Gly Thr Asp Val Ser Leu Ala Arg
95                  100                 105                 110

aat tac acg gta att ctg ggt cac tgt tac tac cat gga cat gta cgg     684
Asn Tyr Thr Val Ile Leu Gly His Cys Tyr Tyr His Gly His Val Arg
            115                 120                 125

gga tat tct gat tca gca gtc agt ctc agc acg tgt tct ggt ctc agg     732
Gly Tyr Ser Asp Ser Ala Val Ser Leu Ser Thr Cys Ser Gly Leu Arg
            130                 135                 140

gga ctt att gtg ttt gaa aat gaa agc tat gtc tta gaa cca atg aaa     780
Gly Leu Ile Val Phe Glu Asn Glu Ser Tyr Val Leu Glu Pro Met Lys
            145                 150                 155

agt gca acc aac aga tac aaa ctc ttc cca gcg aag aag ctg aaa agc     828
Ser Ala Thr Asn Arg Tyr Lys Leu Phe Pro Ala Lys Lys Leu Lys Ser
160                 165                 170

gtc cgg gga tca tgt gga tca cat cac aac aca cca aac ctc gct gca     876
Val Arg Gly Ser Cys Gly Ser His His Asn Thr Pro Asn Leu Ala Ala
175                 180                 185                 190

aag aat gtg ttt cca cca ccc tct cag aca tgg gca aga agg cat aaa     924
Lys Asn Val Phe Pro Pro Pro Ser Gln Thr Trp Ala Arg Arg His Lys
            195                 200                 205

aga gag acc ctc aag gca act aag tat gtg gag ctg gtg atc gtg gca     972
Arg Glu Thr Leu Lys Ala Thr Lys Tyr Val Glu Leu Val Ile Val Ala
            210                 215                 220

gac aac cga gag ttt cag agg caa gga aaa gat ctg gaa aaa gtt aag    1020
Asp Asn Arg Glu Phe Gln Arg Gln Gly Lys Asp Leu Glu Lys Val Lys
            225                 230                 235

cag cga tta ata gag att gct aat cac gtt gac aag ttt tac aga cca    1068
Gln Arg Leu Ile Glu Ile Ala Asn His Val Asp Lys Phe Tyr Arg Pro
        240                 245                 250

ctg aac att cgg atc gtg ttg gta ggc gtg gaa gtg tgg aat gac atg    1116
Leu Asn Ile Arg Ile Val Leu Val Gly Val Glu Val Trp Asn Asp Met
255                 260                 265                 270

gac aaa tgc tct gta agt cag gac cca ttc acc agc ctc cat gaa ttt    1164
Asp Lys Cys Ser Val Ser Gln Asp Pro Phe Thr Ser Leu His Glu Phe
            275                 280                 285

ctg gac tgg agg aag atg aag ctt cta cct cgc aaa tcc cat gac aat    1212
Leu Asp Trp Arg Lys Met Lys Leu Leu Pro Arg Lys Ser His Asp Asn
            290                 295                 300

gcg cag ctt gtc agt ggg gtt tat ttc caa ggg acc acc atc ggc atg    1260
Ala Gln Leu Val Ser Gly Val Tyr Phe Gln Gly Thr Thr Ile Gly Met
            305                 310                 315

gcc cca atc atg agc atg tgc acg gca gac cag tct ggg gga att gtc    1308
Ala Pro Ile Met Ser Met Cys Thr Ala Asp Gln Ser Gly Gly Ile Val
            320                 325                 330
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atg gac cat tca gac aat ccc ctt ggt gca gcc gtg acc ctg gca cat        1356
Met Asp His Ser Asp Asn Pro Leu Gly Ala Ala Val Thr Leu Ala His
335                 340                 345                 350

gag ctg ggc cac aat ttc ggg atg aat cat gac aca ctg gac agg ggc        1404
Glu Leu Gly His Asn Phe Gly Met Asn His Asp Thr Leu Asp Arg Gly
                    355                 360                 365

tgt agc tgt caa atg gcg gtt gag aaa gga ggc tgc atc atg aac gct        1452
Cys Ser Cys Gln Met Ala Val Glu Lys Gly Gly Cys Ile Met Asn Ala
                370         ·       375                 380

tcc acc ggg tac cca ttt ccc atg gtg ttc agc agt tgc agc agg aag        1500
Ser Thr Gly Tyr Pro Phe Pro Met Val Phe Ser Ser Cys Ser Arg Lys
            385                 390                 395

gac ttg gag acc agc ctg gag aaa gga atg ggg gtg tgc ctg ttt aac        1548
Asp Leu Glu Thr Ser Leu Glu Lys Gly Met Gly Val Cys Leu Phe Asn
    400                 405                 410

ctg ccg gaa gtc agg gag tct ttc ggg ggc cag aag tgt ggg aac aga        1596
Leu Pro Glu Val Arg Glu Ser Phe Gly Gly Gln Lys Cys Gly Asn Arg
415                 420                 425             ·   430

ttt gtg gaa gaa gga gag gag tgt gac tgt ggg gag cca gag gaa tgt        1644
Phe Val Glu Glu Gly Glu Glu Cys Asp Cys Gly Glu Pro Glu Glu Cys
                435                 440                 445

atg aat cgc tgc tgc aat gcc acc acc tgt acc ctg aag ccg gac gct        1692
Met Asn Arg Cys Cys Asn Ala Thr Thr Cys Thr Leu Lys Pro Asp Ala
                450                 455                 460

gtg tgc gca cat ggg ctg tgc tgt gaa gac tgc cag ctg aag cct gca        1740
Val Cys Ala His Gly Leu Cys Cys Glu Asp Cys Gln Leu Lys Pro Ala
            465 ·                   470             ·   475

gga aca gcg tgc agg gac tcc agc aac tcc tgt gac ctc cca gag ttc        1788
Gly Thr Ala Cys Arg Asp Ser Ser Asn Ser Cys Asp Leu Pro Glu Phe
    480                 485                 490

tgc aca ggg gcc agc cct cac tgc cca gcc aac gtg tac ctg cac gat        1836
Cys Thr Gly Ala Ser Pro His Cys Pro Ala Asn Val Tyr Leu His Asp
495                 500                 505                 510

ggg cac tca tgt cag gat gtg gac ggc tac tgc tac aat ggc atc tgc        1884
Gly His Ser Cys Gln Asp Val Asp Gly Tyr Cys Tyr Asn Gly Ile Cys
                515                 520                 525

cag act cac gag cag cag tgt gtc aca ctc tgg gga cca ggt gct aaa        1932
Gln Thr His Glu Gln Gln Cys Val Thr Leu Trp Gly Pro Gly Ala Lys
            530                 535                 540

cct gcc cct ggg atc tgc ttt gag aga gtc aat tct gca ggt gat cct        1980
Pro Ala Pro Gly Ile Cys Phe Glu Arg Val Asn Ser Ala Gly Asp Pro
            545                 550                 555

tat ggc aac tgt ggc aaa gtc tcg aag agt tcc ttt gcc aaa tgc gag        2028
Tyr Gly Asn Cys Gly Lys Val Ser Lys Ser Ser Phe Ala Lys Cys Glu
560                 565                 570

atg aga gat gct aaa tgt gga aaa atc cag tgt caa gga ggt gcc agc        2076
Met Arg Asp Ala Lys Cys Gly Lys Ile Gln Cys Gln Gly Gly Ala Ser
575                 580                 585                 590

cgg cca gtc att ggt acc aat gcc gtt tcc ata gaa aca aac atc ccc        2124
Arg Pro Val Ile Gly Thr Asn Ala Val Ser Ile Glu Thr Asn Ile Pro
            595                 600                 605
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ctg cag caa gga ggc cgg att ctg tgc cgg ggg acc cac gtg tac ttg    2172
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ggc gat gac atg ccg gac cca ggg ctt gtg ctt gca ggc aca aag tgt    2220
Gly Asp Asp Met Pro Asp Pro Gly Leu Val Leu Ala Gly Thr Lys Cys
        625             630                 635

gca gat gga aaa atc tgc ctg aat cgt caa tgt caa aat att agt gtc    2268
Ala Asp Gly Lys Ile Cys Leu Asn Arg Gln Cys Gln Asn Ile Ser Val
        640             645                 650

ttt ggg gtt cac gag tgt gca atg cag tgc cac ggc aga ggg gtg tgc    2316
Phe Gly Val His Glu Cys Ala Met Gln Cys His Gly Arg Gly Val Cys
655             660                 665                 670

aac aac agg aag aac tgc cac tgc gag gcc cac tgg gca cct ccc ttc    2364
Asn Asn Arg Lys Asn Cys His Cys Glu Ala His Trp Ala Pro Pro Phe
            675                 680                 685

tgt gac aag ttt ggc ttt gga gga agc aca gac agc ggc ccc atc cgg    2412
Cys Asp Lys Phe Gly Phe Gly Gly Ser Thr Asp Ser Gly Pro Ile Arg
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caa gca gat aac caa ggt tta acc ata gga att ctg gtg acc atc ctg    2460
Gln Ala Asp Asn Gln Gly Leu Thr Ile Gly Ile Leu Val Thr Ile Leu
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tgt ctt ctt gct gcc gga ttt gtg gtt tat ctc aaa agg aag acc ttg    2508
Cys Leu Leu Ala Ala Gly Phe Val Val Tyr Leu Lys Arg Lys Thr Leu
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ata cga ctg ctg ttt aca aat aag aag acc acc att gaa aaa cta agg    2556
Ile Arg Leu Leu Phe Thr Asn Lys Lys Thr Thr Ile Glu Lys Leu Arg
735             740                 745                 750

tgt gtg cgc cct tcc cgg cca ccc cgt ggc ttc caa ccc tgt cag gct    2604
Cys Val Arg Pro Ser Arg Pro Pro Arg Gly Phe Gln Pro Cys Gln Ala
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cac ctc ggc cac ctt gga aaa ggc ctg atg agg aag ccg cca gat tcc    2652
His Leu Gly His Leu Gly Lys Gly Leu Met Arg Lys Pro Pro Asp Ser
            770                 775                 780

tac cca ccg aag gac aat ccc agg aga ttg ctg cag tgt cag aat gtt    2700
Tyr Pro Pro Lys Asp Asn Pro Arg Arg Leu Leu Gln Cys Gln Asn Val
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gac atc agc aga ccc ctc aac ggc ctg aat gtc cct cag ccc cag tca    2748
Asp Ile Ser Arg Pro Leu Asn Gly Leu Asn Val Pro Gln Pro Gln Ser
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act cag cga gtg ctt cct ccc ctc cac cgg gcc cca cgt gca cct agc    2796
Thr Gln Arg Val Leu Pro Pro Leu His Arg Ala Pro Arg Ala Pro Ser
815             820                 825                 830

gtc cct gcc aga ccc ctg cca gcc aag cct gca ctt agg cag gcc cag    2844
Val Pro Ala Arg Pro Leu Pro Ala Lys Pro Ala Leu Arg Gln Ala Gln
                835             840                 845

ggg acc tgt aag cca aac ccc cct cag aag cct ctg cct gca gat cct    2892
Gly Thr Cys Lys Pro Asn Pro Pro Gln Lys Pro Leu Pro Ala Asp Pro
            850                 855                 860

ctg gcc aga aca act cgg ctc act cat gcc ttg gcc agg acc cca gga    2940
Leu Ala Arg Thr Thr Arg Leu Thr His Ala Leu Ala Arg Thr Pro Gly
        865                 870                 875

caa tgg gag act ggg ctc cgc ctg gca ccc ctc aga cct gct cca caa    2988
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Gln Trp Glu Thr Gly Leu Arg Leu Ala Pro Leu Arg Pro Ala Pro Gln
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tat cca cac caa gtg ccc aga tcc acc cac acc gcc tat att aag tga    3036
Tyr Pro His Gln Val Pro Arg Ser Thr His Thr Ala Tyr Ile Lys
895                 900                 905

gaagccgaca cctttttttca acagtgaaga cagaagtttg cactatcttt cagctccagt    3096

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aaggccttat tggaaagcag tcccccaact accccccagct gtgcttatgg taccagatgc    3456

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Leu Trp Asn Gln Gly Arg Ala Asp Glu Val Val Ser Ala Ser Val Arg
        35                  40                  45

Ser Gly Asp Leu Trp Ile Pro Val Lys Ser Phe Asp Ser Lys Asn His
    50                  55                  60

Pro Glu Val Leu Asn Ile Arg Leu Gln Arg Glu Ser Lys Glu Leu Ile
65                  70                  75                  80

Ile Asn Leu Glu Arg Asn Glu Gly Leu Ile Ala Ser Ser Phe Thr Glu
                85                  90                  95

Thr His Tyr Leu Gln Asp Gly Thr Asp Val Ser Leu Ala Arg Asn Tyr
            100                 105                 110

Thr Val Ile Leu Gly His Cys Tyr Tyr His Gly His Val Arg Gly Tyr
        115                 120                 125

Ser Asp Ser Ala Val Ser Leu Ser Thr Cys Ser Gly Leu Arg Gly Leu
    130                 135                 140

Ile Val Phe Glu Asn Glu Ser Tyr Val Leu Glu Pro Met Lys Ser Ala
145                 150                 155                 160

Thr Asn Arg Tyr Lys Leu Phe Pro Ala Lys Lys Leu Lys Ser Val Arg
                165                 170                 175

Gly Ser Cys Gly Ser His His Asn Thr Pro Asn Leu Ala Ala Lys Asn
            180                 185                 190

Val Phe Pro Pro Pro Ser Gln Thr Trp Ala Arg Arg His Lys Arg Glu
        195                 200                 205
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                                page 6

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Thr Leu Lys Ala Thr Lys Tyr Val Glu Leu Val Ile Val Ala Asp Asn
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Arg Glu Phe Gln Arg Gln Gly Lys Asp Leu Glu Lys Val Lys Gln Arg
225               230               235               240

Leu Ile Glu Ile Ala Asn His Val Asp Lys Phe Tyr Arg Pro Leu Asn
                  245               250               255

Ile Arg Ile Val Leu Val Gly Val Glu Val Trp Asn Asp Met Asp Lys
              260               265               270

Cys Ser Val Ser Gln Asp Pro Phe Thr Ser Leu His Glu Phe Leu Asp
          275               280               285

Trp Arg Lys Met Lys Leu Leu Pro Arg Lys Ser His Asp Asn Ala Gln
    290               295               300

Leu Val Ser Gly Val Tyr Phe Gln Gly Thr Thr Ile Gly Met Ala Pro
305               310               315               ·320

Ile Met Ser Met Cys Thr Ala Asp Gln Ser Gly Gly Ile Val Met Asp
              325               330               335

His Ser Asp Asn Pro Leu Gly Ala Ala Val Thr Leu Ala His Glu Leu
          340               345               350

Gly His Asn Phe Gly Met Asn His Asp Thr Leu Asp Arg Gly Cys Ser
          355               360               365

Cys Gln Met Ala Val Glu Lys Gly Gly Cys Ile Met Asn Ala Ser Thr
    370               375               380

Gly Tyr Pro Phe Pro Met Val Phe Ser Ser Cys Ser Arg Lys Asp Leu
385               390               395               400

Glu Thr Ser Leu Glu Lys Gly Met Gly Val Cys Leu Phe Asn Leu Pro
              405               410               415

Glu Val Arg Glu Ser Phe Gly Gly Gln Lys Cys Gly Asn Arg Phe Val
          420               425               430

Glu Glu Gly Glu Glu Cys Asp Cys Gly Glu Pro Glu Glu Cys Met Asn
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Arg Cys Cys Asn Ala Thr Thr Cys Thr Leu Lys Pro Asp Ala Val Cys
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Ala His Gly Leu Cys Cys Glu Asp Cys Gln Leu Lys Pro Ala Gly Thr
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Ala Cys Arg Asp Ser Ser Asn Ser Cys Asp Leu Pro Glu Phe Cys Thr
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Gly Ala Ser Pro His Cys Pro Ala Asn Val Tyr Leu His Asp Gly His
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Ser Cys Gln Asp Val Asp Gly Tyr Cys Tyr Asn Gly Ile Cys Gln Thr
            515             520             525

His Glu Gln Gln Cys Val Thr Leu Trp Gly Pro Gly Ala Lys Pro Ala
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Pro Gly Ile Cys Phe Glu Arg Val Asn Ser Ala Gly Asp Pro Tyr Gly
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Asn Cys Gly Lys Val Ser Lys Ser Ser Phe Ala Lys Cys Glu Met Arg
            565             570             575

Asp Ala Lys Cys Gly Lys Ile Gln Cys Gln Gly Gly Ala Ser Arg Pro
            580             585             590

Val Ile Gly Thr Asn Ala Val Ser Ile Glu Thr Asn Ile Pro Leu Gln
        595             600             605

Gln Gly Gly Arg Ile Leu Cys Arg Gly Thr His Val Tyr Leu Gly Asp
    610             615             620

Asp Met Pro Asp Pro Gly Leu Val Leu Ala Gly Thr Lys Cys Ala Asp
625             630             635             640

Gly Lys Ile Cys Leu Asn Arg Gln Cys Gln Asn Ile Ser Val Phe Gly
            645             650             655

Val His Glu Cys Ala Met Gln Cys His Gly Arg Gly Val Cys Asn Asn
        660             665             670

Arg Lys Asn Cys His Cys Glu Ala His Trp Ala Pro Pro Phe Cys Asp
        675             680             685

Lys Phe Gly Phe Gly Gly Ser Thr Asp Ser Gly Pro Ile Arg Gln Ala
    690             695             700

Asp Asn Gln Gly Leu Thr Ile Gly Ile Leu Val Thr Ile Leu Cys Leu
705             710             715             720

Leu Ala Ala Gly Phe Val Val Tyr Leu Lys Arg Lys Thr Leu Ile Arg
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Leu Leu Phe Thr Asn Lys Lys Thr Thr Ile Glu Lys Leu Arg Cys Val
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Arg Pro Ser Arg Pro Pro Arg Gly Phe Gln Pro Cys Gln Ala His Leu
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Gly His Leu Gly Lys Gly Leu Met Arg Lys Pro Pro Asp Ser Tyr Pro
    770         775                 780

Pro Lys Asp Asn Pro Arg Arg Leu Leu Gln Cys Gln Asn Val Asp Ile
785             790             795                 800

Ser Arg Pro Leu Asn Gly Leu Asn Val Pro Gln Pro Gln Ser Thr Gln
            805             810             815

Arg Val Leu Pro Pro Leu His Arg Ala Pro Arg Ala Pro Ser Val Pro
            820             825             830

Ala Arg Pro Leu Pro Ala Lys Pro Ala Leu Arg Gln Ala Gln Gly Thr
        835             840             845

Cys Lys Pro Asn Pro Pro Gln Lys Pro Leu Pro Ala Asp Pro Leu Ala
    850             855             860

Arg Thr Thr Arg Leu Thr His Ala Leu Ala Arg Thr Pro Gly Gln Trp
865             870             875             880

Glu Thr Gly Leu Arg Leu Ala Pro Leu Arg Pro Ala Pro Gln Tyr Pro
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His Gln Val Pro Arg Ser Thr His Thr Ala Tyr Ile Lys
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15   20   25   30

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Leu Ile Ile Asn Leu Glu Arg Asn Glu Gly Leu Ile Ala Ser Ser Phe
        80   85   90

acg gaa acc cac tat ctg caa gac ggt act gat gtc tcc ctc gct cga   636
Thr Glu Thr His Tyr Leu Gln Asp Gly Thr Asp Val Ser Leu Ala Arg
95   100   105   110

aat tac acg gta att ctg ggt cac tgt tac tac cat gga cat gta cgg   684
Asn Tyr Thr Val Ile Leu Gly His Cys Tyr Tyr His Gly His Val Arg
                115   120   125

gga tat tct gat tca gca gtc agt ctc agc acg tgt tct ggt ctc agg   732
Gly Tyr Ser Asp Ser Ala Val Ser Leu Ser Thr Cys Ser Gly Leu Arg
            130   135   140

gga ctt att gtg ttt gaa aat gaa agc tat gtc tta gaa cca atg aaa   780
Gly Leu Ile Val Phe Glu Asn Glu Ser Tyr Val Leu Glu Pro Met Lys
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agt gca acc aac aga tac aaa ctc ttc cca gcg aag aag ctg aaa agc   828
Ser Ala Thr Asn Arg Tyr Lys Leu Phe Pro Ala Lys Lys Leu Lys Ser
160   165   170

gtc cgg gga tca tgt gga tca cat cac aac aca cca aac ctc gct gca   876
Val Arg Gly Ser Cys Gly Ser His His Asn Thr Pro Asn Leu Ala Ala
175   180   185   190

aag aat gtg ttt cca cca ccc tct cag aca tgg gca aga agg cat aaa   924
Lys Asn Val Phe Pro Pro Pro Ser Gln Thr Trp Ala Arg Arg His Lys
                195   200   205

aga gag acc ctc aag gca act aag tat gtg gag ctg gtg atc gtg gca   972
Arg Glu Thr Leu Lys Ala Thr Lys Tyr Val Glu Leu Val Ile Val Ala
            210   215   220

gac aac cga gag ttt cag agg caa gga aaa gat ctg gaa aaa gtt aag   1020
Asp Asn Arg Glu Phe Gln Arg Gln Gly Lys Asp Leu Glu Lys Val Lys
            225   230   235

cag cga tta ata gag att gct aat cac gtt gac aag ttt tac aga cca   1068
Gln Arg Leu Ile Glu Ile Ala Asn His Val Asp Lys Phe Tyr Arg Pro
        240   245   250

ctg aac att cgg atc gtg ttg gta ggc gtg gaa gtg tgg aat gac atg   1116
Leu Asn Ile Arg Ile Val Leu Val Gly Val Glu Val Trp Asn Asp Met
255   260   265   270

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gac aaa tgc tct gta agt cag gac cca ttc acc agc ctc cat gaa ttt      1164
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            275             280             285

ctg gac tgg agg aag atg aag ctt cta cct cgc aaa tcc cat gac aat      1212
Leu Asp Trp Arg Lys Met Lys Leu Leu Pro Arg Lys Ser His Asp Asn
            290             295             300

gcg cag ctt gtc agt ggg gtt tat ttc caa ggg acc acc atc ggc atg      1260
Ala Gln Leu Val Ser Gly Val Tyr Phe Gln Gly Thr Thr Ile Gly Met
            305             310             315

gcc cca atc atg agc atg tgc acg gca gac cag tct ggg gga att gtc      1308
Ala Pro Ile Met Ser Met Cys Thr Ala Asp Gln Ser Gly Gly Ile Val
            320             325             330

atg gac cat tca gac aat ccc ctt ggt gca gcc gtg acc ctg gca cat      1356
Met Asp His Ser Asp Asn Pro Leu Gly Ala Ala Val Thr Leu Ala His
335             340             345             350

gag ctg ggc cac aat ttc ggg atg aat cat gac aca ctg gac agg ggc      1404
Glu Leu Gly His Asn Phe Gly Met Asn His Asp Thr Leu Asp Arg Gly
            355             360             365

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Cys Ser Cys Gln Met Ala Val Glu Lys Gly Gly Cys Ile Met Asn Ala
            370             375             380

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Ser Thr Gly Tyr Pro Phe Pro Met Val Phe Ser Ser Cys Ser Arg Lys
            385             390             395

gac ttg gag acc agc ctg gag aaa gga atg ggg gtg tgc ctg ttt aac      1548
Asp Leu Glu Thr Ser Leu Glu Lys Gly Met Gly Val Cys Leu Phe Asn
            400             405             410

ctg ccg gaa gtc agg gag tct ttc ggg ggc cag aag tgt ggg aac aga      1596
Leu Pro Glu Val Arg Glu Ser Phe Gly Gly Gln Lys Cys Gly Asn Arg
415             420             425             430

ttt gtg gaa gaa gga gag gag tgt gac tgt ggg gag cca gag gaa tgt      1644
Phe Val Glu Glu Gly Glu Glu Cys Asp Cys Gly Glu Pro Glu Glu Cys
            435             440             445

atg aat cgc tgc tgc aat gcc acc acc tgt acc ctg aag ccg gac gct      1692
Met Asn Arg Cys Cys Asn Ala Thr Thr Cys Thr Leu Lys Pro Asp Ala
            450             455             460

gtg tgc gca cat ggg ctg tgc tgt gaa gac tgc cag ctg aag cct gca      1740
Val Cys Ala His Gly Leu Cys Cys Glu Asp Cys Gln Leu Lys Pro Ala
            465             470             475

gga aca gcg tgc agg gac tcc agc aac tcc tgt gac ctc cca gag ttc      1788
Gly Thr Ala Cys Arg Asp Ser Ser Asn Ser Cys Asp Leu Pro Glu Phe
            480             485             490

tgc aca ggg gcc agc cct cac tgc cca gcc aac gtg tac ctg cac gat      1836
Cys Thr Gly Ala Ser Pro His Cys Pro Ala Asn Val Tyr Leu His Asp
495             500             505             510

ggg cac tca tgt cag gat gtg gac ggc tac tgc tac aat ggc atc tgc      1884
Gly His Ser Cys Gln Asp Val Asp Gly Tyr Cys Tyr Asn Gly Ile Cys
            515             520             525

cag act cac gag cag cag tgt gtc aca ctc tgg gga cca ggt gct aaa      1932
Gln Thr His Glu Gln Gln Cys Val Thr Leu Trp Gly Pro Gly Ala Lys
            530             535             540
```

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```
cct gcc cct ggg atc tgc ttt gag aga gtc aat tct gca ggt gat cct      1980
Pro Ala Pro Gly Ile Cys Phe Glu Arg Val Asn Ser Ala Gly Asp Pro
        545                 550                 555

tat ggc aac tgt ggc aaa gtc tcg aag agt tcc ttt gcc aaa tgc gag      2028
Tyr Gly Asn Cys Gly Lys Val Ser Lys Ser Ser Phe Ala Lys Cys Glu
        560                 565                 570

atg aga gat gct aaa tgt gga aaa atc cag tgt caa gga ggt gcc agc      2076
Met Arg Asp Ala Lys Cys Gly Lys Ile Gln Cys Gln Gly Gly Ala Ser
575                 580                 585                 590

cgg cca gtc att ggt acc aat gcc gtt tcc ata gaa aca aac atc ccc      2124
Arg Pro Val Ile Gly Thr Asn Ala Val Ser Ile Glu Thr Asn Ile Pro
                595                 600                 605

ctg cag caa gga ggc cgg att ctg tgc cgg ggg acc cac gtg tac ttg      2172
Leu Gln Gln Gly Gly Arg Ile Leu Cys Arg Gly Thr His Val Tyr Leu
            610                 615                 620

ggc gat gac atg ccg gac cca ggg ctt gtg ctt gca ggc aca aag tgt      2220
Gly Asp Asp Met Pro Asp Pro Gly Leu Val Leu Ala Gly Thr Lys Cys
        625                 630                 635

gca gat gga aaa atc tgc ctg aat cgt caa tgt caa aat att agt gtc      2268
Ala Asp Gly Lys Ile Cys Leu Asn Arg Gln Cys Gln Asn Ile Ser Val
        640                 645                 650

ttt ggg gtt cac gag tgt gca atg cag tgc cac ggc aga ggg gtg tgc      2316
Phe Gly Val His Glu Cys Ala Met Gln Cys His Gly Arg Gly Val Cys
655                 660                 665                 670

aac aac agg aag aac tgc cac tgc gag gcc cac tgg gca cct ccc ttc      2364
Asn Asn Arg Lys Asn Cys His Cys Glu Ala His Trp Ala Pro Pro Phe
                675                 680                 685

tgt gac aag ttt ggc ttt gga gga agc aca gac agc ggc ccc atc cgg      2412
Cys Asp Lys Phe Gly Phe Gly Gly Ser Thr Asp Ser Gly Pro Ile Arg
            690                 695                 700

caa gca gaa gca agg cag gaa gct gca gag tcc aac agg gag cgc ggc      2460
Gln Ala Glu Ala Arg Gln Glu Ala Ala Glu Ser Asn Arg Glu Arg Gly
            705                 710                 715

cag ggc cag gag ccc gtg gga tcg cag gag cat gcg tct act gcc tca      2508
Gln Gly Gln Glu Pro Val Gly Ser Gln Glu His Ala Ser Thr Ala Ser
720                 725                 730

ctg aca ctc atc tga gccctcccat gacatggaga ccgtgaccag tgctgctgca      2563
Leu Thr Leu Ile
735

gaggaggtca cgcgtcccca aggcctcctg tgactggcag cattgactct gtggctttgc      2623

catcgtttcc atgacaacag acacaacaca gttctcgggg ctcaggaggg gaagtccagc      2683

ctaccaggca ggtctgcaga aacagtgcaa ggaagggcag cgacttcctg gttgagcttc      2743

tgctaaaaca tggacatgct tcagtgctgc tcctgagaga gtagcaggtt accactctgg      2803

caggccccag ccctgcagca aggaggaaga ggactcaaaa gtctggcctt tcactgagcc      2863

tccacagcag tgggggagaa gcaagggttg ggcccagtgt cccctttccc cagtgacacc      2923

tccgccttgg cagccctgat gactggtctc tggctgcaac ttaatgctct gatatggctt      2983

ttagcattta ttatatgaaa atagcagggt tttagttttt aatttatcag agaccctgcc      3043

acccattcca tctccatcca agcaaactga atggcattga aacaaactgg agaagaaggt      3103
```

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```
aggagaaagg gcggtgaact ctggctcttt gctgtggaca tgcgtgacca gcagtactca    3163

ggtttgaggg tttgcagaaa gccagggaac ccacagagtc accaaccctt catttaacaa    3223

gtaagaatgt taaaaagtga aaacaatgta agagcctaac tccatccccc gtggccatta    3283

ctgcataaaa atagagtgca ttttgaaata aaaaaaaaaa aaaaa                     3328
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<210> 4

<211> 738

<212> PRT

<213> Homo sapiens

<400> 4

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Met Ala Ala Arg Pro Leu Pro Val Ser Pro Ala Arg Ala Leu Leu Leu
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Ala Leu Ala Gly Ala Leu Leu Ala Pro Cys Glu Ala Arg Gly Val Ser
                20              25                  30


Leu Trp Asn Glu Gly Arg Ala Asp Glu Val Val Ser Ala Ser Val Arg
            35                  40                  45


Ser Gly Asp Leu Trp Ile Pro Val Lys Ser Phe Asp Ser Lys Asn His
        50              55                  60


Pro Glu Val Leu Asn Ile Arg Leu Gln Arg Glu Ser Lys Glu Leu Ile
65                  70                  75                  80


Ile Asn Leu Glu Arg Asn Glu Gly Leu Ile Ala Ser Ser Phe Thr Glu
                85                  90                  95


Thr His Tyr Leu Gln Asp Gly Thr Asp Val Ser Leu Ala Arg Asn Tyr
                100                 105                 110


Thr Val Ile Leu Gly His Cys Tyr Tyr His Gly His Val Arg Gly Tyr
            115                 120                 125


Ser Asp Ser Ala Val Ser Leu Ser Thr Cys Ser Gly Leu Arg Gly Leu
        130                 135                 140


Ile Val Phe Glu Asn Glu Ser Tyr Val Leu Glu Pro Met Lys Ser Ala
145                 150                 155                 160


Thr Asn Arg Tyr Lys Leu Phe Pro Ala Lys Lys Leu Lys Ser Val Arg
                165                 170                 175


Gly Ser Cys Gly Ser His His Asn Thr Pro Asn Leu Ala Ala Lys Asn
                180                 185                 190
```

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```
Val Phe Pro Pro Pro Ser Gln Thr Trp Ala Arg Arg His Lys Arg Glu
        195             200             205

Thr Leu Lys Ala Thr Lys Tyr Val Glu Leu Val Ile Val Ala Asp Asn
    210             215             220

Arg Glu Phe Gln Arg Gln Gly Lys Asp Leu Glu Lys Val Lys Gln Arg
225             230             235             240

Leu Ile Glu Ile Ala Asn His Val Asp Lys Phe Tyr Arg Pro Leu Asn
            245             250             255

Ile Arg Ile Val Leu Val Gly Val Glu Val Trp Asn Asp Met Asp Lys
            260             265             270

Cys Ser Val Ser Gln Asp Pro Phe Thr Ser Leu His Glu Phe Leu Asp
        275             280             285

Trp Arg Lys Met Lys Leu Leu Pro Arg Lys Ser His Asp Asn Ala Gln
    290             295             300

Leu Val Ser Gly Val Tyr Phe Gln Gly Thr Thr Ile Gly Met Ala Pro
305             310             315             320

Ile Met Ser Met Cys Thr Ala Asp Gln Ser Gly Gly Ile Val Met Asp
            325             330             335

His Ser Asp Asn Pro Leu Gly Ala Ala Val Thr Leu Ala His Glu Leu
            340             345             350

Gly His Asn Phe Gly Met Asn His Asp Thr Leu Asp Arg Gly Cys Ser
    355             360             365

Cys Gln Met Ala Val Glu Lys Gly Gly Cys Ile Met Asn Ala Ser Thr
    370             375             380

Gly Tyr Pro Phe Pro Met Val Phe Ser Ser Cys Ser Arg Lys Asp Leu
385             390             395             400

Glu Thr Ser Leu Glu Lys Gly Met Gly Val Cys Leu Phe Asn Leu Pro
            405             410             415

Glu Val Arg Glu Ser Phe Gly Gly Gln Lys Cys Gly Asn Arg Phe Val
            420             425             430

Glu Glu Gly Glu Glu Cys Asp Cys Gly Glu Pro Glu Glu Cys Met Asn
    435             440             445

Arg Cys Cys Asn Ala Thr Thr Cys Thr Leu Lys Pro Asp Ala Val Cys
    450             455             460
```

```
Ala His Gly Leu Cys Cys Glu Asp Cys Gln Leu Lys Pro Ala Gly Thr
465                 470                 475                 480

Ala Cys Arg Asp Ser Ser Asn Ser Cys Asp Leu Pro Glu Phe Cys Thr
                485                 490                 495

Gly Ala Ser Pro His Cys Pro Ala Asn Val Tyr Leu His Asp Gly His
            500                 505                 510

Ser Cys Gln Asp Val Asp Gly Tyr Cys Tyr Asn Gly Ile Cys Gln Thr
            515                 520                 525

His Glu Gln Gln Cys Val Thr Leu Trp Gly Pro Gly Ala Lys Pro Ala
    530                 535                 540

Pro Gly Ile Cys Phe Glu Arg Val Asn Ser Ala Gly Asp Pro Tyr Gly
545                 550                 555                 560

Asn Cys Gly Lys Val Ser Lys Ser Ser Phe Ala Lys Cys Glu Met Arg
            565                 570                 575

Asp Ala Lys Cys Gly Lys Ile Gln Cys Gln Gly Gly Ala Ser Arg Pro
            580                 585             .   590

Val Ile Gly Thr Asn Ala Val Ser Ile Glu Thr Asn Ile Pro Leu Gln
        595                 600                 605

Gln Gly Gly Arg Ile Leu Cys Arg Gly Thr His Val Tyr Leu Gly Asp
    610                 615                 620

Asp Met Pro Asp Pro Gly Leu Val Leu Ala Gly Thr Lys Cys Ala Asp
625                 630                 635                 640

Gly Lys Ile Cys Leu Asn Arg Gln Cys Gln Asn Ile Ser Val Phe Gly
            645                 650                 655

Val His Glu Cys Ala Met Gln Cys His Gly Arg Gly Val Cys Asn Asn
            660                 665                 670

Arg Lys Asn Cys His Cys Glu Ala His Trp Ala Pro Pro Phe Cys Asp
        675                 680                 685

Lys Phe Gly Phe Gly Gly Ser Thr Asp Ser Gly Pro Ile Arg Gln Ala
    690                 695                 700

Glu Ala Arg Gln Glu Ala Ala Glu Ser Asn Arg Glu Arg Gly Gln Gly
705                 710                 715                 720

Gln Glu Pro Val Gly Ser Gln Glu His Ala Ser Thr Ala Ser Leu Thr
                725                 730                 735

Leu Ile
                        page 15
```

<210> 5

<211> 2642

<212> DNA

<213> Homo sapiens

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cattgaaaaa ctaaggtgtg tgcgcccttc ccggccaccc cgtggcttcc aaccctgtca     180
ggctcacctc ggccaccttg gaaaaggcct gatgaggaag ccgccagatt cctacccacc     240
gaaggacaat cccaggagat tgctgcagtg tcagaatgtt gacatcagca gacccctcaa     300
cggcctgaat gtccctcagc cccagtcaac tcagcgagtg cttcctcccc tccaccgggc     360
cccacgtgca cctagcgtcc ctgccagacc cctgccagcc aagcctgcac ttaggcaggc     420
ccaggggacc tgtaagccaa accccctca gaagcctctg cctgcagatc tctggccag     480
aacaactcgg ctcactcatg ccttggccag accccagga caatgggaga ctgggctccg     540
cctggcaccc ctcagacctg ctccacaata tccacaccaa gtgcccagat ccacccacac     600
cgcctatatt aagtgagaag ccgacacctt ttttcaacag tgaagacaga agtttgcact     660
atctttcagc tccagttgga gttttttgta ccaacttta ggattttttt taatgtttaa     720
aacatcatta ctataagaac tttgagctac tgccgtcagt gctgtgctgt gctatggtgc    780
tctgtctact tgcacaggta cttgtaaatt attaatttat gcagaatgtt gattacagtg     840
cagtgcgctg tagtaggcat ttttaccatc actgagtttt ccatggcagg aaggcttgtt     900
gtgctttag tattttagtg aacttgaaat atcctgcttg atgggattct ggacaggatg     960
tgtttgcttt ctgatcaagg ccttattgga aagcagtccc ccaactaccc ccagctgtgc    1020
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gtcaatcaag tctgtttact gcaaggttca acttattaac aattaggcag actctttatg    1680
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cttgcaaaaa ctacaaccaa tggaatgtga tgttcatggg tatagttcat gtctgctatc   1740

attattcgta gatattggac aaagaacctt ctctatgggg catcctcttt ttccaacttg   1800

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caacctacct gttgagcatc acagaatgtg ataaggaaat caacttgctt atcaacttcc   1920

taaatattat gagatgtggc ttgggcagca tccccttgaa ctcttcactc ttcaaatgcc   1980

tgactaggga gccatgtttc acaaggtctt taaagtgact aatggcatga gaaatacaaa   2040

aatactcaga taaggtaaaa tgccatgatg cctctgtctt ctggactggt tttcacatta   2100

gaagacaatt gacaacagtt acataattca ctctgagtgt tttatgagaa agccttcttt   2160

tggggtcaac agttttccta tgctttgaaa cagaaaaata tgtaccaaga atcttggttt   2220

gccttccaga aaacaaaact gcatttcact ttcccggtgt tccccactgt atctaggcaa   2280

catagtattc atgactatgg ataaactaaa cacgtgacac aaacacacac aaaagggaac   2340

ccagctctaa tacattccaa ctcgtatagc atgcatctgt ttattctata gttattaagt   2400

tctttaaaat gtaaagccat gctggaaaat aatactgctg agatacatac agaattactg   2460

taactgatta cacttggtaa ttgtactaaa gccaaacata tatatactat taaaaaggtt   2520

tacagaattt tatggtgcat tacgtgggca ttgtcttttt agatgcccaa atccttagat   2580

ctggcatgtt agcccttcct ccaattataa gaggatatga accaaaaaaa aaaaaaaaaa   2640

aa                                                                  2642
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<210> 6

<211> 205

<212> PRT

<213> Homo sapiens

<400> 6

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Asp Asn Gln Gly Leu Thr Ile Gly Ile Leu Val Thr Ile Leu Cys Leu
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Leu Ala Ala Gly Phe Val Val Tyr Leu Lys Arg Lys Thr Leu Ile Arg
                20                  25                  30


Leu Leu Phe Thr Asn Lys Lys Thr Thr Ile Glu Lys Leu Arg Cys Val
            35                  40                  45


Arg Pro Ser Arg Pro Pro Arg Gly Phe Gln Pro Cys Gln Ala His Leu
        50                  55                  60


Gly His Leu Gly Lys Gly Leu Met Arg Lys Pro Pro Asp Ser Tyr Pro
65                  70                  75                  80


Pro Lys Asp Asn Pro Arg Arg Leu Leu Gln Cys Gln Asn Val Asp Ile
```

Ser Arg Pro Leu Asn Gly Leu Asn Val Pro Gln Pro Gln Ser Thr Gln
            100   105   110

Arg Val Leu Pro Pro Leu His Arg Ala Pro Arg Ala Pro Ser Val Pro
            115   120   125

Ala Arg Pro Leu Pro Ala Lys Pro Ala Leu Arg Gln Ala Gln Gly Thr
    130   135   140

Cys Lys Pro Asn Pro Pro Gln Lys Pro Leu Pro Ala Asp Pro Leu Ala
145   150   155   160

Arg Thr Thr Arg Leu Thr His Ala Leu Ala Arg Thr Pro Gly Gln Trp
            165   170   175

Glu Thr Gly Leu Arg Leu Ala Pro Leu Arg Pro Ala Pro Gln Tyr Pro
            180   185   190

His Gln Val Pro Arg Ser Thr His Thr Ala Tyr Ile Lys
        195   200   205

<210> 7

<211> 908

<212> DNA

<213> Homo sapiens

<400> 7

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agaccgtgac cagtgctgct gcagaggagg tcacgcgtcc ccaaggcctc ctgtgactgg     180
cagcattgac tctgtggctt tgccatcgtt tccatgacaa cagacacaac acagttctcg     240
gggctcagga ggggaagtcc agcctaccag gcaggtctgc agaaacagtg caaggaaggg     300
cagcgacttc ctggttgagc ttctgctaaa acatggacat gcttcagtgc tgctcctgag     360
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aacttaatgc tctgatatgg ctttttagcat ttattatatg aaaatagcag ggttttagtt     600
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tgaaacaaac tggagaagaa ggtaggagaa agggcggtga actctggctc tttgctgtgg     720
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gtcaccaacc cttcatttaa caagtaagaa tgttaaaaag tgaaaacaat gtaagagcct     840
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                                    page 18

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      aactccatcc cccgtggcca ttactgcata aaaatagagt gcattttgaa ataaaaaaaa    900

      aaaaaaaa                                                            908
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<210> 8
<211> 34
<212> PRT
<213> Homo sapiens

<400> 8

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Glu Ala Arg Gln Glu Ala Ala Glu Ser Asn Arg Glu Arg Gly Gln Gly
1               5                   10                  15

Gln Glu Pro Val Gly Ser Gln Glu His Ala Ser Thr Ala Ser Leu Thr
            20                  25                  30

Leu Ile
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<210> 9
<211> 23
<212> DNA
<213> Homo sapiens

<400> 9
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cctgtgacct cccagagttc tgc                                             23
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<210> 10
<211> 23
<212> DNA
<213> Homo sapiens

<400> 10
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gaattcctat ggttaaacct tgg                                             23
```

<210> 11
<211> 24
<212> DNA
<213> Homo sapiens

<400> 11

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cttgctgccg gatttgtggt ttat                                              24
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<210> 12

<211> 24

<212> DNA

<213> Homo sapiens

<400> 12
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<210> 13

<211> 23

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (15)..(15)

<223> Mismatch (g instead of c)

<400> 13
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<210> 14

<211> 23

<212> DNA

<213> Homo sapiens

<400> 14
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ggactctgca gcttcctgcc ttg                                               23
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<210> 15

<211> 21

<212> DNA

<213> Homo sapiens

<400> 15
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tcggggctca ggaggggaag t                                                 21
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<210> 16

<211> 21

<212> DNA

<213> Homo sapiens

<400> 16
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<210> 17

<211> 24

<212> DNA

<213> Homo sapiens

<400> 17
ggccgggtga tctttggtct cttc   24

<210> 18

<211> 24

<212> DNA

<213> Homo sapiens

<400> 18
cccggctgtc tgtcttggtg ctct   24

<210> 19

<211> 21

<212> DNA

<213> Homo sapiens

<400> 19
ctgctgagat gggaaagggc t   21

<210> 20

<211> 21

<212> DNA

<213> Homo sapiens

<400> 20
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